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Trends in Production and Utilization of Fish Silage

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Decreased availability of fish for fishmeal production due to the increase in human consumption of low value fish and diversification of fish exports necessitates a second look at the fish processing wastes as a potential raw material. The quantum of fish processing wastes, though considerable, are not available centrally making the production of fishmeal uneconomical. Fish silage offers a practical alternative towards conversion of such wastes into feed for aquaculture and animal husbandry. The production of both the acid added and fermented types of fish silages has seen a number of changes in the recent years. Attempts have been made to curtail excessive hydrolysis of proteins by heating and use of formalin. Co-drying of silage with solid binders has made its storage and handling easier as has the concentration of the centrifuged silage. The presence of high amounts of polyunsaturated fatty acids in fish silage is advantageous in aquaculture. The utilization of fish silage as animal feed has met with success in most animals except poultry. Fish silage has been used extensively as a feed in aquaculture for a number of fish and shellfish with a promising potential in the field of larval nutrition.

Keywords : Fish silage, Wastes, Feeds, Animals, Aquaculture.

The signs of over-exploitation in fisheries are visible worldwide by several indicators. The decline in marine fish catches and catch per unit effort, growth over fishing in general and in specific fisheries all attest to the phenomenon (Pillai 1995; Uozumi 1995; Mace 1997; FAO 1999). Thanks to the explosive growth of aquaculture, the demand for fishmeal in aquafeeds is increasing and currently utilize 15% of the global fishmeal production (New 1996). On the other hand, caught between the increasing exports of quality fish (MPEDA 1998) and burgeoning demand for fishmeal for animal feed and aquaculture, the consumption of formerly unutilized or underutilized fish species has increased. Fishmeal production worldwide has shown a stagnating/declining trend (Table 1), and in India, it has been stagnant at ~ 0.4 million tonnes from 1992 to 1998 (Anon 1997a)

The occasional necessity to convert seasonal excess catches or poor quality catches into fishmeal cannot form the basis of an industry and as such, reduction plants for fishmeal are few along the Indian coast. Most fishmeal is produced by size-reduction of sun-dried by-catch and other waste materials. The only raw materials that are regularly available, but currently unutilized, are the fish processing wastes from factories. Indian export of processed seafood in 1998-99 was about 422,329 tonnes valued at US $1625 million (Anon 1999). Even assuming a conservative estimate of 10% average wastes during production of chilled, frozen, canned and dried fish, this translates to about 27,929 tonnes of wastes. This figure excludes wastes generated in countless retail markets, for which no estimates are available, but are likely to be even higher. However, the quantum of waste generated per factory or retail market is too small to set up fishmeal plants, and centralized collection of wastes is often impractical due to their dispersed location and high transportation costs. On the other hand, these quantities can and do cause disposal problems for the processors and retail markets, especially in the monsoon. However, a potential use can be found for the problem if these wastes are utilized for the production of fish silage.

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Silage production

Fish silage is made by either addition of acid to whole fish or parts of fish, or by *in-situ* generation of acid using lactic acid bacteria. The added acid(s) can be inorganic, organic or a mixture of both. Such silages are usually called acid-preserved silages (AS) in contrast to the fermented silages (FS) which are made by mixing fish / fish wastes with a fermentable sugar source like molasses and a starter culture of Lactic Acid Bacteria (LAB).

Acid preserved silage (AS)

Both inorganic and organic acids can be used for making fish silage, but the latter are preferred, as smaller quantities are required, thus offsetting their higher costs (Raa and Gildberg 1982). About 2.8-6.3% (w/w) of concentrated sulphuric acid is required for oily or bony fish in comparison to 2.2-3% (w/w) of organic acids. Although sulphuric acid is cheaper in comparison to organic acids, the greater quantities required and the problem of neutralising the silages before use as feed, discourage its use. Phosphoric acid also has been used for making fish silage, but has to be combined with potassium sorbate (0.1%) as preservative to prevent mold and yeast growth (Levin et al. 1989).

TABLE 1. WORLD FISH UTILIZATION BY REDUCTION

	Year					
	1990	1992	1994	1995	1996	1997
Utilization *	28.19	29.29	33.47	30.78	30.39	29.50

Source : FAO 1999 * Million tonnes

TABLE 2. COMPOSITION OF DIFFERENT LAYERS OF AN ACID ENSILED FISH SILAGE (%, ON AS IS BASIS)

Silage layer	Fractions, %	Moisture, %	Crude protein, %	Ash, %	Crude lipid, %
Whole silage	100.0	79.8	11.5	1.6	5.37
Oil layer	3.7	1.8	ND	0.17	98.0
Emulsified layer	4.9	59.0	14.6	1.5	32.5
Aqueous layer	67.4	85.4	10.8	1.45	0.2
Sediment	17.8	76.4	16.1	2.3	4.5

Source : Raghunath and McCurdy (1987)

Acids used in fish silage exert their preservative action by the passage of the undissociated acid molecule into the bacterial cell where it dissociates and lowers the pH to kill the cell. Thus, organic acids with higher pKa like propionic/ formic acid (pKa 4.86 and 3.75, respectively) are more effective than inorganic acids as a greater proportion of their molecules are undissociated at higher pH. Trash fish have been preserved with 2.2% (w/w) of formic or propionic acids or a 1.1% (w/w) each mixture of both (Austreng and Aasgaard 1986), but more commonly a concentration of 2.5 to 3% is used for safety (Raa and Gildberg 1982). For materials with a high mineral content, even 8% (v/w) of a 1 : 1 v/v mixture of formic and propionic acids may be necessary (Ariyani and Buckle 1991). As organic acids can be rather expensive, a 3+1(v+v) mixture of sulphuric and formic acids also can be used at 3% (w/w) level, with sulphuric acid being replaced by hydrochloric or phosphoric acid, if necessary (Raa and Gildberg 1982, Mahendrakar et al. 1991b). Less commonly, sulphuric-acetic and phosphoric-citric acid combinations have also been used (Viana et al. 1993).

Acid preserved fish silages liquefy rather quickly and after about a week in tropics, up to 80% of the proteins become solubilized (Raa and Gildberg 1982). The liquefied silage separates into 3-4 layers; an oily layer at top, sometimes with an underlying emulsified layer, an aqueous middle layer containing the liquefied proteins and a sediment or sludge containing undigested protein, scales, bones, etc (Raghunath and McCurdy 1987). Compositions of these layers are shown in Table 2. The liquefaction/digestion of fish are due to the action of a wide variety of endogenous proteinases present in the fish. The ratio of non-protein nitrogen (NPN) to total nitrogen (TN) in the final silage can vary from 42 (Jl et al. 1996) to 90% (Haaland and Njaa 1989), depending on the extent of protein breakdown in which pH plays a major role (Raghunath and McCurdy 1990). Although a high conversion of proteins to small peptides and free amino acids promotes better liquefaction and separation of aqueous and lipid components, it can be disadvantageous because: (a) Aromatic amino acids separate from the aqueous phase due to their low solubility (Raa and Gildberg 1982) and may even crystallize out (Espe et al. 1991). (b) Higher leaching losses can occur when such silages are incorporated into feeds due to their greater solubility (Lopez and Viana 1995) and (c) Intact proteins appear to be utilised better in feeds, especially fish (Stone et al. 1989).

Because of these reasons, a number of attempts have been made to curtail the hydrolysis of proteins in AS either by heating to inactivate the proteinases responsible (Viana et al. 1993; Lopez and Viana 1995) or by addition of formalin (Husain and Offer 1987, Offer and Husain 1987; Fagbenro and Jauncey 1994). Even extracts of ginger and potato have been tried with some encouraging results, but formalin reportedly reduced digestibility in animals fed such silages (Fagbenro and Jauncey 1994).

The liquefied silages when used for feeding are either co-dried or mixed with other dry ingredients like soy-meal, gluten, wheat bran, rice bran, etc., to make their handling easier (Mahendrakar et al. 1991a; Ali et al. 1994). Alternatively in Denmark and Norway, the aqueous phase after centrifugal separation from the oil is concentrated (Hansen and Alsted 1986; Skrede 1988; Lie et al. 1988) to gain benefits of lowered transportation costs, bulk and ease of storage. Co-ensilation of trash fish discards with soybean meal at 10 and 40% levels and added acid has also been tried (Balogun and Dabrowki 1993), which apparently improved the nutritional quality.

Fish and fish wastes are rich in polyunsaturated fatty acids. As high as 59% of the total lipids in a skipjack tuna canning waste silage was reported to be unsaturated, with EPA and DHA constituting about 33% of the lipids (Jl et al. 1996). Even special silages have been made using fish neural tissues (tuna eyes) rich in highly unsaturated fatty acids for use as enrichers to rotifers and Artemia in the nutrition of larval marine fish (Tocher et al. 1997). But their oxidation may create problems of rancidity in fish silage, which can be prevented by fat extraction or addition of antioxidants (Hall and Ledward 1986).

Fermented silages (FS)

The production of fermented fish silages depends on *in situ* production of lactic acid by lactic acid bacteria, which are added to the fish along with a fermentable carbohydrate source. Although lactic acid bacteria are present naturally in fish (Nair et al. 1997), their numbers are very few to compete against other microflora and start a lactic fermentation. Hence, an external inoculum of lactic acid bacteria is necessary. The various species of lactic acid bacteria used in fish silage are

:*Lactobacillus plantarum* (Viete and Bello 1992; Ottati and Bello 1992a,b; Fagbenro and Jauncey 1993, 1994, 1995; Lassen 1995b), Yoghurt bacteria like *L. bulgaricus* and *Streptococcus thermophilus* (Areche et al. 1992, Yoon et al. 1997), *L. delbruecki* spp *bulgaricus* and *S.salivarius* spp *thermophilus* (Martinez-Valdivieso et al. 1996), a marine yeast, *Hansenula montevideo* (Bertullo 1992) and natural inocula like sauerkraut (fermented cabbage) (Dhatemwa 1989).

L. plantarum appears to be the most effective starter culture as per a comparative trial conducted to evaluate three different lactics (Bello et al. 1992). Lassen (1995a), who experimented with10^7 to 10^9 cfu/g silage levels of *L. plantarum*, concluded that a level of 10^8 cfu/g was ideal for a stable low pH and high lactic acid production.

The fermentable carbohydrate (FC) source most often used in fish silage is molasses, which is used at 10-15% level (Fagbenro and Jauncey 1994, 1995; Sachindra et al. 1994), but sucrose (Areche et al. 1992) and sugarcane (Ali et al. 1985) have also been tried as FC source. Dry molasses when used as the FC source at 5% for crab wastes and crop residues did not result in satisfactory fermentation (Fontenot et al. 1992), while 5% dextrose with inoculum of *L. plantarum* at 10^8 cfu/g silage for fish offal resulted in satisfactory fermentation (Lassen 1995 a, b). Some workers have reportedly used 5% salt in fermented silages to reduce protein hydrolysis and swelling of the fermenting mass (Fagbenro and Jauncey 1993; Ahmed and Mahendrakar 1995).

Pre-acidification of the fish-molasses mixture to ensure a smooth lactic fermentation has been tried by some workers (Sachindra et al. 1994) using acetic and lactic acids, which did not affect the final pH (Lassen 1995b). Fermentation proceeds satisfactorily between 20-35^{0}C, but is slowed down at lower temperatures (Fagbenro and Jauncey 1993). Using yoghurt bacteria at 40^{0}C, enough acidity could be produced by 48 h. (Areche et al. 1992). The time taken in FS to produce a stable and desired pH is usually longer than for AS, but optimal conditions for fermentation can reduce the period required to comparable levels. A reduction of pH from 6.8 to 4.3 in one week with 2-3.2% of lactic acid production was reported by Lassen (1995a). The extent of protein breakdown is normally lesser in FS when compared to AS (Raa and Gildberg 1982) and a NPN/TN ratio of only 51% was attained even after 180 days (Fagbenro and Jauncey 1993). Heating of the silage to arrest or minimize proteolysis has been tried in fermented silages also (Fagbenro and Jauncey 1993), while preservatives like sorbic acid have sometimes been used as adjuncts (Ottati and Bello 1992 a, b).

Fish silage in animal feeding and aquaculture

Fish silage made by acid addition or lactic fermentation has been used in feeding various farm animals like pigs, calves, fox, mink, sheep, beef cattle and poultry. Fish silage is mainly used to replace the traditional diets of soya flour or fishmeal. The more recent of these studies in this direction are summarised in Table 3 and an excellent account of earlier experiments can be found in the review by Raa and Gildberg (1982). Acid silages made with inorganic acids like sulphuric acid need to be neutralised before using as feed, which can be a limitation. Inclusion of fish silage at high levels in finishing stages are reported to impair performance and more significantly produce off-flavours in the meat (Raa and Gildberg 1982, Mahendrakar et al. 1991a) due to the fish lipids. However, in recent studies such effects have not been observed (Fontenot et al. 1992).

However, the performance of poultry broilers has been consistently poor when fed on fish silage, the reasons for which are not clear. Thiamine deficiency, use of spoiled raw material, oxidation of fish lipids etc, have been put forward as the possible reasons (Raa and Gildberg 1982) for the poor performance. Interestingly, such poor performances have been usually observed with broilers and it is not known whether the same is the case with layers as well.

In the last 10 years, fish silage has been extensively tested as a feed ingredient for many fish and shellfish in aquaculture. These studies are summarised in Table 4. It can be seen that with few exceptions, fish silage whether fermented or acid preserved has been as good as or better than other control protein sources such as fish or soy meal. The process of ensilation has also been extended to Poultry viscera and used for feeding in aquaculture (Jagannatha Rao et al. 1996). A variety of freshwater and marine fish have been raised with fish silage with no apparent ill effects and

TABLE 3. RESPONSE OF FARMED ANIMALS FED WITH FISH SILAGE

Animals used in study	Response	Reference
Pigs	Satisfactory, normal average daily gain, feed intake and feed to gain ratio	Avdalov et al. (1992) Ottati and Bello, (1992a,b); Areche et al(1992); Myer et al. (1990)
Calves	Easy acceptance, good weight increase, food consumption and digestibility	Viete and Bello (1992)
	Depressed feed intake and live weight gain	Offer and Hussain (1987)
Beef cattle	Fed along with cellulosic material efficient utilization and no effect on taste of meat	Fontenot et al (1992)
Sheep	Same as above Better performance with fermented rather than acid preserved silage	Fontenot et al (1992) Abzinge (1986)
Poultry (broilers)	Lower feed conversion	Guevara et al (1992)
	Lower feed conversion, loss of appetite, leg weakness and thinner plumage	Bigueras-Benitez and Nacorda (1992)
	Poor performance, broiler carcass quality reduced	Ol Oghobo et al (1988)
Mink and foxes	No ill effects seen even on feeding of stored silages	Espe and Haaland (1992); Skrede (1988)

TABLE 4. FEEDING STUDIES IN AQUACULTURE WITH FISH SILAGE

Fish/Shell fish used in the study	Control protein source	Level at which fish silage used & source	Performance	Reference
Abalone Juveniles, (*Halotis fulgens*)	Kelp, *Macrocystis pyrifera*	30% protein Fish and abalone viscera silage	53-61 μm/day growth vs 1.5 to 18 μm/day	Viana et al (1996)
African catfish (*Chrysichthys nigrodigitatus*)	Commercial fishmeal	38% protein, a) cooked tuna waste AS	Equal to or better than control	Cisse et al (1995)
		b) cooked sardine, FS	Equal to or better than control	Cisse et al (1995)
Black cachama (*Colossoma macropomum*)	Fishmeal	18% protein	No difference to control, FCR 1.34:1 vs 1.37:1	Valencia - Ramos et al (1994)
Common carp (*Cyprinus carpio*)	Fishmeal	8% FS or FPVS, Mash and pellet forms, 30.8% protein	Better growth with both fermented silages	Jagannatha Rao et al (1996)
Tialpia (*Oreochromis niloticus*)		FS	High ADC	Fagbenro and Jauncey (1995)
	Soy/poultry by-product/ feather/meat bonemeal	30% protein, 50% replacement of control by FS	Similar FCR, PER and ADC to that of control; Carcass composition and hepato-somatic index unaffected.	Fagbenro (1994)
	Fishmeal	1:1 and 1:3 Fishmeal: FS	1:1 Blend & control more efficient, highest PER withh 1:1	Lapie and Biguera-Benitez (1992)
Mrigal, (*Cirrhinus mrigala*)	Fishmeal and wheat bran	35% protein, AS; 50 to 100% replacement of control	75% replacement better than control	Ali et al (1994)
Atlantic Salmon (*Salmo solar*)	Ground herrring	100% herring or Dogfish silage	No apparent decrease in palatability, WG, FE & PER for large fish Lower performance with smaller fish. No sensory difference in fillets	Heras et al (1994)
Atlantic Salmon (*Salmo solar*) herrring	Fresh herrring/ meal	FS	No difference in fillet flavours between silage and control diets	Parish et al (1991)
Atlantic Salmon (*Salmo solar*) adults	Dry pellet feed	100%, partly or non defatted silage concentrate	No difference between diets in FC, PER and PPV	Lie et al (1988)
Tilapia (*O. niloticus*) and catfish (*Clarias gariepinus*)	Fishmeal	FS & soy meal blend, 25-75% replacement of FM	No difference between control in FC, PER, PPV. APD increased with increase in FS+SBM blend in diet	Fagbenro et al (1994)
Cat fish (*Clarias gariepinus*)	Fishmeal	19 - 41% FS in diet	14% FM diet better than 41% FS diet	Ayinla and Akande (1988)
	Unknown	1:1 w/w blend of FS with SBM	No difference in APDC of different silage diets	Fagbenro and Jauncey (1993)
Hybrid striped bass (*Morone saxatilis*)	Unknown	Freeze dried silage	Significant improvement in larval growth	Gallagher (1993)
Eel, (*Anguilla* sp.) fingerlings	Fish & meat meal	10 - 20% protein from silage	Better FCR & PER with silage diets	Goncalves et al (1989)
Seabass, (*Lates calcarifer*)	Live preys	Silage, fresh fish meat; with and without vitamins	Lowest growth rate and production	Lawonyawut (1987)

APD - Apparent protein digestibility, APDC - Apparent protein digestibility coefficient, AS - Acid silage, FCR - Feed conversion ratio, FM - Fishmeal, FS - Fermented fish silage, FPVS - Fermented poultry viscera silage, PER - Protein efficiency ratio, PPV - Protein productive value, SBM - Soybean meal, WG - Weight gain

the growth performance has been equal to or better than control groups. Fish silage diets seem to have an advantage in the crucial early stages of aquaculture as better larval growth and survival rate have been reported with hybrid striped bass and seabass respectively (Table 4). The stability and nutrient leaching of silage incorporated feeds also were good when binders were used (Fagbenro and Jauncey 1995). Thus, fish silage appears to be a promising feed ingredient in aquaculture.

Fish silage vs. Fishmeal : Merits and drawbacks

Fish silage has several advantages over fishmeal. It is generally a better preferred option of waste utilization in small operations (Anon 1991) and works well on large or small fishing vessels or even in small isolated places with little fish production, which is economically not feasible for a fishmeal plant (Arason et al. 1990). The scale of its production can be varied at will without affecting the economy of the process (Raa and Gildberg 1982). The energy requirement of fish silage is very small when compared to fishmeal and it is easily produced in an artisanal manner with low cost and minimal equipment (Villela et al. 1992). On a larger scale, converted tankers of 2000 ton capacity have been used to produce fish silage from factory fish wastes in Norway (Anon 1997b).

Fishmeal is always a suspected source of pathogens especially of Salmonellosis when used in feeds. Several pathogenic bacteria like *Aeromonas salmonicida, Mycobacterium chelonei, Yersenia ruckeri, Renibacterium salmoninarum* are rapidly destroyed under acid silage conditions, while a few resistant viral pathogens like the infectious pancreatic necrosis virus can be inactivated by pasteurization or by virucidal agents (Smail et al. 1990; Whipple and Rohovec 1994). Silages either wet or dried have lower bacterial counts than freshly prepared fishmeal, but dried fish silage upon storage have been reported to have higher bacterial counts (Hoq et al. 1995).

Prawn processing wastes upon ensilation, contain value added nutrients like carotenoid pigments and n-3 polyunsaturated fatty acids (Guillou et al. 1995), which are released by ensilation, but are otherwise difficult to extract even by solvents. Fish silage once made, by acid or fermented processes can be co-dried along with other feed ingredients like soymeal, poultry by-product meal (Fagbenro 1994) or carbohydrate fillers and sundried openly without fly infestation which is impossible to escape in fishmeal (Raa and Gildberg 1982). Such co-dried products have low aerobic counts and water activity (Dong et al. 1993).

While the production of fishmeal from fatty fish requires more capital equipment for cooking, pressing and oil separation, fish offal, fatty or lean fish can all be ensiled and the oil layer easily separated by decanting or centrifugation. The oil from fish silage is rich in polyunsaturated fatty acids (Jl et al. 1996) and although oxidation problems are encountered (Hall and Ledward 1986), they can be overcome by the addition of antioxidants (Dong et al. 1993; Ahmed and Mahendrakar 1996).

The disadvantages of fish silage are that it is more bulky and difficult to transport than fishmeal. Concentration as practiced in Norway and Denmark (Skrede 1988; Anon 1988) or its co-drying with other feed ingredients as discussed earlier can be a way of overcoming this problem. The thiaminase activity in fish can persist in silage leading to losses of thiamine, but mixing the thiaminolytic silage with a dry binder meal into a moist pellet is shown to suspend thiamine destruction (Anglesea and Jackson 1985). Corrosion of metallic surfaces, especially when inorganic acids are used in acid silages may pose a problem during storage. However, when organic acids are used in conjunction with low cost plastic containers, or with fermented silages, problems of corrosion have not been reported.

In conclusion, fish silage is an easy way of converting fish wastes from by-catches and processing industry into a valuable feed ingredient, which consumes less energy than fishmeal and is particularly suited where small quantities of wastes are available. While the use of fish silage as feed in animal husbandry has been well accepted with the exception of broilers, its use in aquaculture is growing and no drawbacks are reported so far.

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RESEARCH PAPER

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Proximate Composition, Minerals, Tannins, *In Vitro* Protein Digestibility and Effect of Cooking on Protein Fractions of Hyacinth Bean (*Dolichos lablab*)

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Four hyacinth bean cultivars (*Dolichos lablab* L.) were used in this study. Protein contents varied from 22.7-26.1%, fat contents 1.1-2.0%, moisture contents 7.8-8.1%, ash contents 3.8-4.3%, fibre contents 9.3-10.9% and carbohydrate contents 48.3-53.6%. Phosphorus values ranged from 305-312, magnesium : 167.4-231.1, sodium : 6-9 and potassium : 183.3-214 mg/100g. Tannin contents of the four hyacinth bean cultivars were in the range of 201-353 mg/100g, while the *in vitro* protein digestibility of the four cultivars was in the range 59.2-62.0%. The variation in protein fractions was : albumin 1.8-2.5%, globulin 69.9-81.2%, prolamin 2.7-4.4%, G_1-glutelins 0.8-1.8%, G_2-glutelins 1.1-3.0%, G_3-glutelins 10.0-11.9% and insoluble proteins 0.2-9.9%. Two hyacinth bean cultivars, selected for their higher albumin and globulin fractions, were cooked in 150 ml of boiling distilled water under reflex. The protein fractions of the cooked seeds were determined. Results indicated that albumin and globulin fractions decreased significantly ($p<0.05$) for both cultivars after cooking. This decrease was accompanied by a significant increase in prolamin, G_1-glutelins, G_2-glutelins and G_3-glutelins fractions.

Keywords : Cooking, Hyacinth bean, Minerals, Protein digestibility, Protein fractions, Proximate composition, Tannins, *Dolichos lablab*.

Legumes are good sources of proteins, minerals, B-vitamins, dietary fibre and complex carbohydrates. They are also rich in many antinutrients such as trypsin inhibitors, tannins, phytates and hemaglutannins or lectins, which decrease digestibility of nutrients or cause toxicity when taken in large concentrations (FAO 1982).

Hyacinth bean (*Dolichos lablab* L.) also known as bonavist bean is cultivated in many tropical and semitropical areas and is an important food in many parts of Asia. The mature seeds are often used dehulled as dhal in Asia or as substitute for broad beans in fried bean cake in Africa. Deshpande et al (1982) reported that tannin contents of whole and dehulled *Phaseolus vulgaris* ranged from 33.7-282.8 and 10-28.7 mg catechin equivalents/100 g, respectively. They found that removal of seed coat lowered the tannin content of beans by 68-95%. The seed coat colour of the grain was associated with tannin content (Harris et al 1970). Bullard et al. (1982) and Deshpande et al (1982) found insignificant correlation between tannin content and seed coat colour. Blessani et al (1963) reported on the contribution of non-tannin compounds to pigmentation of seed coat. Deka and Sarkar (1990) reported that tannin of five cultivars of mature seeds of *Dolichos lablab* ranged from 2000-2205 mg/100g. The susceptibility of native proteins to proteolysis by mammalian digestive enzymes is an important factor which contributes to the nutritive value of seed storage proteins. Shastry and John (1991) reported a value of 65% for the *in vitro* protein digestibility of hyacinth bean. Liener (1976) reported values of 57% and 82% for digestibility of raw and heated hyacinth bean respectively. Digestibility values were found to be 70, 59, 79, and 88% for soybeans, pigeon peas, cowpeas and lentils, respectively (FAO 1982).

According to Landry and Moureaux (1970) seed protein could be separated into six fractions (albumin, globulin, prolamin, G_1-glutelins, G_2-glutelins and G_3-glutelins) by selective extraction procedure. Each fraction tends to have different characteristics and amino acid composition, the relative proportion of each strongly affects the level of particular amino acids in the total grain protein (Johnson and Lay 1974). For many years, traditional processing practices have been followed to convert grain legumes into a consumable form. The desirable effect of heating on legumes can be attributed to loss of toxicity of proteinaceous anti-nutrient and easier digestion of heated denatured globulins, which were normally resistant to denaturation and digestion in native state (Bressani 1985).

The objective of the present study was to quantify differences in proximate composition, mineral content, tannin content and protein digestibility of four hyacinth bean cultivars, and to reveal the proportions of protein solubility fractions and to study the effect of cooking on those fractions.

Materials and Methods

Four hyacinth bean cultivars : 'A-1', 'A-6', 'A,-5' and 'A-2' obtained from Department of Agronomy, Faculty of Agriculture, Shambat, Sudan were used in this study. The grains were cleaned, freed from foreign material and ground to pass 0.4 mm mesh screen.

Proximate composition : Crude protein and fat were determined according to Pearson (1970). Moisture, fibre and ash contents were determined according to AOAC (1984) and carbohydrates were calculated by difference.

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Minerals : Sodium and potassium were determined according to AOAC (1984). Magnesium, calcium and phosphorous were determined according to Chapman and Pratt (1961).

Tannins and in vitro protein digestibility : Tannins were determined according to the method of AOAC (1970). *In vitro* protein digestibility was assessed according to the method of Maliwal (1983) as modified by Manjula and John (1991).

Protein fractionation : Protein fractions of hyacinth bean were determined by the method of Landry and Moureaux (1970; Table 1).

Cooking of hyacinth bean : Cooking of the samples was carried out by cooking one hundred seeds which were placed into 150 ml boiled distilled water and boiled until half of seeds were split.

Statistical analysis : Each determination was carried out in triplicate samples and the figures were then averaged. Data were assessed by analysis of variance (ANOVA) (Snedecor and Cochran 1987) and by Duncan's multiple range test with probability $p \leq 0.05$ (Duncan 1955).

TABLE 1. PROTEIN EXTRACTION PROCEDURE

Step	Extractants	Time of extraction min.	Fraction
1	NaCl. 0.5 M 4°C	60,30,30	I(Globulin)
2	Water 4°C	15,15	II(Albumin
3	EtOH 60% 20°C	30,30	III(Prolamin)
	and then at 60°C	30	
	2PrOH 55% 20°C	60,30,15	
4	EtOH 60%+2ME 0.6(v/v)20°C	30,30	IV(G_1-glutelins)
	2PrOH 55%+2ME 0.6(v/v)20°C	30,30	
5	NaCl, 0.5M, pH10+2ME 0.6(v/v)20°C	60,30,15	V(G_2-glutelins)
6	$NaDodSO_4$0.5%(w/v) pH10+2ME 0.6(v/v) 20_0C	60,30,15	VI(G_3-glutelins)

Results and Discussion

Proximate composition : The proximate composition of hyacinth bean seeds is shown in Table 2. The results are expressed as on dry matter basis (DMB). Protein content was in the range of 22.7-26.1%. There was a significant difference ($p \leq 0.05$) in protein contents among 'A-1', 'A-2' and 'A-6' compared to 'A-5' cultivar. Values obtained in this study were higher than those obtained by El Hardallou and El Tinay (1985), who reported an average value of 20%. The results were in agreement with those by Shastry and John (1991) who reported 23%. The results were also in agreement with those reported by Deka and Sarkar (20-31%; 1990). Values were also similar to those obtained by Khattab (24%; 1970).

Dietary fibre contents of the four cultivars ranged from 9.3-10.9%. The results obtained in this study were higher than those of Deka and Sarkar (1990) and El Hardallou and El Tinay (1985) who reported values in the range of 7.6-9.6% and an average of 7.7%.

The data indicated that 'A-6' cultivar had the highest fat content of 1.9%, while 'A-2' cultivar had the lowest fat content (1.0%). The value obtained in this study was similar to that (1.2%) obtained by El Hardallou and El Tinay (1985). Total ash of the four cultivars ranged from 3.7-4.3%. These values were close to the value of 3.6% reported by El Hardallou and El Tinay (1985). Carbohydrate content varied from 48.3-53.6%.

Minerals : Data on mineral content is shown in Table 3. The cultivars were relatively higher in phosphorus, potassium and calcium. Phosphorus content of 'A-1', 'A-6', 'A-5' and 'A-2' was 305, 312, 306 and 305 mg/100g, respectively. These values were less than those reported by Deka and Sarkar (1990), whose values were in the range of 388-483 mg/100g.

Potassium content of 'A-1' cultivar was significantly ($p \leq 0.05$) higher than those of 'A-5' and 'A-6'. El Tinay et al (1988) reported 1247, 1497, 933, 1119 and 1718 mg/100g for potassium content of chickpea, white bean, lentil, broad bean and soybean, respectively.

Calcium content ranged from 231.1 for 'A-5' cultivar to 167.4 mg/100g for 'A-1' cultivar. Very little amount of sodium was observed in hyacinth bean cultivars. The values were 6.1 for 'A-5' cultivar and 9 mg/100g for 'A-1' cultivar.

'A-1' cultivar had the highest content of magnesium with 48.6, while 'A-2' had the lowest one with 38.7 mg/100 g. 'A-6' and 'A-5' cultivars had 45.1 and 42.8 mg/100 g, respectively. The differences in Mg content among the cultivars were

TABLE 2. PROXIMATE COMPOSITION OF FOUR HYACINTH BEAN CULTIVARS

Cultivar	Seed coat colour	Moisture, %	Ash, %	Fibre,%	Fat, %	Protein, %	Carbohydrate, %
'A-1'	White	8.1 ± 0.27^a	4.3 ± 0.03^a	9.3 ± 0.28^a	1.2 ± 0.06^a	26.1 ± 0.18^a	51.0 ± 0.24^a
'A-6'	Golden	8.0 ± 0.06^a	3.8 ± 0.05^b	9.3 ± 0.70^a	1.9 ± 0.03^a	24.7 ± 0.09^a	52.4 ± 0.59^a
'A-5	Dark brown	7.9 ± 0.43^a	3.8 ± 0.13^b	10.9 ± 1.36^a	1.1 ± 0.10^a	22.7 ± 2.0^b	53.6 ± 2.0^a
'A-2'	Black	7.8 ± 0.06^a	3.7 ± 0.10^b	10.7 ± 0.76^a	1.0 ± 0.56^a	25.0 ± 0.09^a	48.3 ± 0.9^a
Overall mean		8.0	3.9	10.5	1.3	24.6	51.3

Values are means (± SD)

Means not sharing a common superscript letter in column are significantly different at $p \leq 0.05$ as assessed by Duncan's Multiple - Range Test

TABLE 3. MINERAL CONTENT (MG/100G) OF FOUR HYACINTH BEAN CULTIVARS

Cultivar	K	Na	Ca	Mg	P
'A-1'	214 ± 4.58[a]	9.0 ± 1.0[a]	167.4 ± 1.2[d]	48.6 ± 6.08[a]	305 ± 5.00[a]
'A-6'	187 ± 6.8[c]	8.9 ± 2.0[a]	178.6 ± 1.0[c]	45.1 ± 2.15[b]	312 ± 2.00[a]
'A-5'	185 ± 4.16[c]	6.1 ± 0.0[a]	231 ± 2.4[a]	42.8 ± 0.00[c]	306.3 ± 4.4[a]
'A-2'	208 ± 4.0[ab]	8.0 ± 1.0[a]	187 ± 2.5[b]	38.7 ± 1.04[d]	305 ± 1.0[a]
Overall mean	198.6	8.0	191.1	43.8	307

Values are means (±SD)

Means not sharing a common superscript letter in a column are significantly different at $p \leq 0.05$ as assessed by Duncan's Multiple-Range Test

significant. Magnesium contents of 178, 178, 114, 189 and 271 mg/100 g for chickpea, white bean, lentil, broad bean and soybean, respectively were reported by El Tinay et al (1988).

Tannins content and in vitro protein digestibility : The tannin content and the *in vitro* protein digestibility (IVPD) are listed in Table 4. The tannin content ranged from 353.3 for 'A-5' cultivar to 201.7 mg/100 g for 'A-1' cultivar. There was a significant difference ($p \leq 0.05$) between 'A-1' cultivar (white) and 'A-2' (black), 'A-5' (dark brown) and 'A-6'(golden). Elias et al (1979) have observed that white seed coat cultivars of field bean had lower tannin content than those with red or dark seed coats. Jambunathan and Singh (1980) have found that pigeon pea varieties with red seed coats had higher tannin content than those with white seed coats. In the present study 'A-1' cultivar with white seed coat had very low tannins compared to the other colored cultivars. However, in 'A-2' (black), 'A-5' (dark brown) and 'A-6' (golden) no association between the seed coat colour and tannin content was observed. This is similar to the observation of Price et al (1980) who did not find association between the colour of the seed coat and tannin content in varieties of cowpea, chickpea, pigeon pea and mung bean. Tannin contents reported in this study were less than those (2205 mg/100 g) obtained by Deka and Sarkar (1990) for *Dolichos lablab*.

TABLE 4. TANNIN CONTENT (MG/100G) AND *IN VITRO* PROTEIN DIGESTIBILITY (IVPD) OF FOUR HYACINTH BEAN CULTIVARS

Cultivar	Seed coat Colour	Tannin content (mg/100g)	IVPD %
'A-1'	White	201.7±2.36[b]	60.6±0.3[b]
'A-6'	Golden	353±16.1[a]	59.2±0.3[c]
'A-5'	Dark brown	353.3±12.47[a]	62.0±0.5[a]
'A-2'	Black	343.3±37.7[a]	60.7±0.3[b]
Overall mean		313	60.6

Values are means (±SD)

Means not sharing a common superscipt letter in a column are significantly different at $p \leq 0.05$ as assessed by Duncan's Multiple-Range Test

The IVPD was in the range 59.2-62.0%. Differences were significant between 'A-5', 'A-2' and 'A-6' while the difference between 'A-2' and 'A-1' was insignificant. Values were lower than those obtained by Shastry and John (1991).

Protein fractions : The percentages of the protein fractions of the hyacinth bean cultivar were shown in Table 5. The globulin fraction was found to be in highest amount (81.2%) in 'A-6' cultivar, while the lowest amount (69.85%) was in 'A-2' cultivar. The albumin was 2.5% in 'A-5' cultivar, while in 'A-6' it was 1.76%. Data obtained here for albumin and globulin fractions were higher than those reported by Shastry and John (1991) who reported that albumins and globulins constitute 64% of the total seed protein of hyacinth bean. The highest level of prolamin appeared in 'A-5' (4.41%), while the lowest one was in 'A-1'. G_1-glutelin fractions of hyacinth bean cultivars ranged from 1.79% in 'A-2' to 0.75% in 'A-1', while the G_2-glutelins and G_3-glutelins ranged from 3.03% to 1.12% and from 11.86% to 9.99%, respectively. The insoluble protein fractions for the four hyacinth bean cultivars was in the range of 9.89% for 'A-2' cultivar to 0.22% for 'A-6' cultivar.

Effect of cooking on protein fractions : Two cultivars of hyacinth bean were chosen according to their high content of albumin and globulin; they were cooked and the protein fractions were determined (Table 6). The albumin and globulin fractions, of the cooked samples, decreased significantly ($p \leq 0.05$) from 2.2% to 1.7% and from 78.6% to 7.9% respectively for the 'A-1' cultivar, while the decrease in the same fractions was from 1.8% and 81.2% to 0.9% and 4.6% respectively for the 'A-6' cultivar. Reduction was noted in the storage proteins of cooked cowpea seeds as reported by Nugdalla and El Tinay (1997). On the other hand, the prolamin, G_1-glutelins, G_2-glutelins and G_3-glutelins fractions were significantly increased after cooking of both cultivars. The results obtained in this study were similar to those obtained by Lakshmi and Nadi (1979) who reported that sesame α-glutelins undergo dissociation, denaturation and aggregation upon heating. Heating at 100°C in SDS solution, usually in the presence of thiols, completely dissociate polypeptide chains from one another (Lakshmi and Nadi 1979). This

TABLE 5. PROTEIN FRACTIONS OF FOUR HYACINTH BEAN CULTIVARS

Cultivar	Total protein, %	Globulin, %	Albumin, %	Prolmin, %	G_1-glutelin, %	G_2-glutelin, %	G_3-glutelin, %	Insoluble protein, %	Total protein recovered %
'A-1'	26.08 ± 0.18^{a}	78.58 ± 0.65^{a}	2.18 ± 0.08^{a}	2.66 ± 0.58^{a}	0.75 ± 0.58^{a}	1.12 ± 0.29^{a}	10.76 ± 0.73^{a}	3.90 ± 0.36^{cd}	99.94
'A-6'	24.68 ± 0.1^{ac}	81.20 ± 0.2^{a}	1.76 ± 0.52^{a}	3.97 ± 1.72^{a}	1.09 ± 0.24^{b}	3.03 ± 0.2^{a}	10.74 ± 1.37^{a}	0.22 ± 0.07^{b}	102
'A-5'	22.66 ± 2.01^{d}	75.83 ± 0.47^{b}	2.51 ± 0.11^{a}	4.41 ± 0.47^{a}	1.17 ± 0.07^{c}	1.51 ± 0.31^{a}	11.86 ± 1.05^{a}	4.05 ± 0.75^{d}	101.35
'A-2'	25.02 ± 0.09^{a}	69.85 ± 0.05^{c}	2.14 ± 0.14^{a}	3.15 ± 0.34^{a}	1.79 ± 0.25^{a}	2.2 ± 0.39^{a}	9.99 ± 1.43^{a}	9.89 ± 0.10^{a}	99
Overall mean	24.61	76.37	2.15	3.55	1.20	1.97	10.84	4.51	

Values are means (± SD)

Means not sharing a common superscript letter in a column are significantly different at $p \leq 0.05$ as assessed by Duncan's Multiple-Range Test

TABLE 6. EFFECT OF COOKING ON PROTEIN FRACTIONS OF HYACINTH BEAN CULTIVARS 'A-1' AND 'A-6'

Cultivar	Treatment	Total protein, %	Globulin, %	Albumin, %	Prolamin, %	G_1-glutelin, %	G_2-glutelin, %	G_3-glutelin, %	Insoluble protein, %	Total protein recovered%
'A-1'	Uncooked	26.08 ± 0.18^{a}	78.58 ± 0.65^{a}	2.18 ± 0.7^{a}	2.66 ± 0.58^{a}	0.75 ± 0.1^{a}	1.12 ± 0.29^{a}	10.76 ± 0.73^{a}	3.90 ± 0.36^{a}	99.94
	Cooked	26.01 ± 0.28^{a}	7.89 ± 0.58^{b}	1.56 ± 0.13^{b}	5.00 ± 0.39^{b}	5.79 ± 0.82^{b}	8.28 ± 0.23^{b}	62.49 ± 1.46^{b}	3.38 ± 0.15^{a}	94.40
'A-6'	Uncooked	24.68 ± 0.08^{a}	81.20 ± 0.2^{a}	1.76 ± 0.52^{a}	3.97 ± 1.72^{a}	1.09 ± 0.24^{a}	3.03 ± 0.20^{a}	10.74 ± 1.36^{a}	0.22 ± 0.07^{b}	102.00
	Cooked	23.45 ± 0.0^{b}	4.60 ± 0.21^{b}	0.87 ± 0.15^{b}	5.71 ± 1.76^{a}	1.93 ± 0.44^{a}	9.00 ± 0.44^{b}	70.97 ± 1.66^{a}	6.04 ± 0.15^{b}	99.12

Vlaues are means (± SD)

Means not sharing a common superscript letter in a coloumn are significantly different at $P \leq 0.05$ as assessed by Duncan's Multiple-Range Test

explains the increase in low molecular weight fractions in cooked samples and results in decreasing the high molecular weight fractions through cleavage of disulphide linkages.

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RESEARCH PAPER

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Effect of Immobilization and Irradiation on Pigment Production by *Monascus ruber* ATCC 8111

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Attempts were made to increase pigment production by *Monascus ruber* ATCC 8111, using mutation and immobilization. Immobilized beads prepared by entrapment of 20 g % wet mycelium in 2% calcium alginate, followed by curing for 10 days at room temperature (250 immobilized bead/50 ml fermentation medium). On the other hand, 150 immobilized spore bead gave maximum yield of pigment. A semi-continuous production of pigment using immobilized mycelium for 4 cycles led to two-fold increase in the pigment output. Ultraviolet (UV) irradiated spores of *Monascus ruber* ATCC 8111 showed a two-fold increase in the pigment yield after 5 min exposure as compared to the wild type.

Keywords : *Monascus ruber*, Immobilization, Irradiation, Pigment production.

Literature survey indicated scant work on the immobilization process for the production of the pigment by *Monascus ruber.* Alginate, the major structural polysaccharide of marine brown algae, in the presence of divalent cations, especially calcium cause gelation. Since gel formation can take place under mild conditions, entrapment in this matrix is suitable for immobilization of viable cells (Marek et al. 1985). Evans and Wang (1984) studied pigment production by immobilized *Monascus ruber* using calcium alginate and a polymeric adsorbent resin. They concluded that the addition of this resin to the immobilized *Monascus* culture increased the pigment yield and production rate over those of the free cell fermentation.

Irradiation of wild *Monascus* strains by UV light, neutron- or X- rays, or combination of these methods can result in mutants with advantageous properties such as rapid growth, superior pigment production and elimination of ascospore formation or albino mutants (Lin and Suen 1973; Hiroi et al. 1979; Wong and Bau 1978). Wong and Koehler (1981) worked on a mutant, *Monascus purpureus* GI which was isolated from a wild type, transferred several times after ultraviolet irradiation. This new strain was extremely dark red in colour and also released more pigment into the yeast extract culture medium. Lin (1973) reported that *Monascus ruber* F-2 an effective pigment - productive strain in submerged culture was improved by N-methyl-N - nitro- nitrosoguanidine (NTG) treatment; Su yuan-chi et al (1973) reported red Koji processing to be improved by using an ultraviolet ray - induced mutant of *Monascus anka.*

This study was focused on immobilization of spores and mycelia of *Monascus ruber* ATCC 8111 for semicontinuous production of the pigment. The use of UV irradiated strain is also considered.

Materials and Methods

Spore immobilization : This was carried out as described by Dutta et al (1993). Spores (10ml) were added to 2% sodium alginate solution. The alginate spores mixture was added dropwise by a narrow glass tube to 2% calcium chloride solution, to obtain fine pellets diameter of 3-5 mm. The beads so obtained were allowed to cure at room temperature for about 2 h, separated and then washed with 50 ml sterile distilled water. Different number of these beads (50, 100, 150, 200, 250 and 300) were transferred under aseptic conditions to previously sterilized 250 ml Erlenmeyer flasks containing 50 ml peptone broth medium and incubated using a reciprocal shaker (200 rpm) at 30^{0}C for 10 days.

Mycelium immobilization : The cell entrapment in calcium alginate beads was carried out according to Maddox et al (1981), Schmauder et al (1991) and Schlosser et al (1993), where 20 g wet weight mycelium was added to sterile 100 ml 2% sodium alginate solution. The alginate - cell mixture was added dropwise using a narrow glass tube to 2% calcium chloride solution and continued as mentioned under spore immobilization.

Effect of different alginate concentrations : Different concentrations (1,2,3 and 4%) of alginate were used to prepare 200 immobilized beads. Analysis of the red pigments was carried out after 10 days incubation of the fungus under submerged conditions (200 rpm and 30^{0}C).

Effect of different mycelium wet weights : Different wet weights (5, 10, 20 and 30 g) of the fungal mycelia were filtered under aseptic conditions and added to 100 ml of 2% alginate solution; 200 immobilized mycelial beads were gathered from each weight and added to the fermentation medium and incubated for 10 days at 30^{0}C as mentioned earlier.

Effect of different shaking periods : Immobilization process was repeated using 20 g wet mycelia in 2% alginate as described previously; 200 beads were gathered aseptically, added to the fermentation medium and incubated under shaking condition at 30^{0}C and 200 rpm for different time intervals, i.e. 5, 10, 15, 20 and 25 days.

Effect of immobilized mycelial beads number Immobilization was carried out by filtering 20 g wet mycelium, which was added to 2% sodium alginate. Different numbers of these immobilized beads (50-300) were added to glucose-peptone fermentation medium and incubated for 10 days at 30^{0}C as mentioned earlier.

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Effect of different numbers of immobilized spores : (50-300) beads were added to glucose - peptone fermentation medium and incubated for 10 days at 200 rpm and 30^{0}C.

Semicontinuous production of red pigment : At the end of each fermentation cycle of 10 days, the calcium alginate beads were aseptically separated from the fermentation broth by filtration on sterile funnel. The beads were washed twice with sterile water and resuspended in 250 ml Erlenmeyer flasks containing 50 ml peptone broth medium and the red pigment content was determined in the filtrate. The sequence of 10 days fermentation, bead separation and washing was repeated for 4 cycles. At the end of the 4th cycle, the amount of pigment was determined in the homogenized beads.

Mutant for Monascus pigment production : Experiments with ultraviolet irradiation were done to improve the yield of pigment production by *Monascus ruber* ATCC 8111 (Hiroi et al. 1979). *Monascus* was cultured on peptone agar medium : peptone, 2%; glucose, 10%, $CaCl_2$ 0.1%; KH_2 PO_4 0.1%, Mg SO_4, 0.5% and agar, 2% in 100 ml distilled water (Wong and Koehler 1981). Conidia of *Monascus* were allowed to form on agar slant with this media for 10 days at 30^{0}C, diluted to about 500 conidia /ml with sterile water and irradiated with UV (100 V, 13 W) from a distance of 40 cm for 1,3,5,7 and 10 min. On the basis of diameter and pigmentation of the giant colony, mutants induced by UV - irradiation were selected and transferred to peptone agar slants for 10 days at 30^{0}C (Hiroi et al. 1979). The spore suspension of these slants was used to inoculate 250 ml Erlenmeyer flasks containing 50 ml sterile peptone broth medium. After 10 days incubation period at 30^{0}C, pigment analysis was carried out to evaluate pigment concentration.

Estimation of pigments : Cultures (50 ml) were homogenized in a Sorvall Omnimixer homogenizer chamber for 30 s. 0.5 ml aliquots of the homogenate placed in a test tube was diluted with 9.5 ml of 0.9% sodium chloride solution and 10 ml of absolute ethanol. The mixture was shaken thoroughly for several seconds, then allowed to stand for 15 min. before being shaken again. The mixture was then filtered to remove the extracted mycelia. The extract was placed in al cm quartz cuvette and measured at 495 nm (Carels and Shepherd 1977)

Results and Discussion

Optimization of the immobilization conditions was investigated in order to improve pigment production of the immobilized living cells. Calcium alginate beads (2%) showed the highest production of pigment (Fig 1), which decreased on either side of this level. This was thought to be due to diffusional resistance of the pigments through alginate beads and also due to the difficulty in stimulating the nutrients by the fungus through high concentrations of alginate.

Mycelium weights also affected pigment production by immobilized cells. Twenty grams wet weight gave the highest amount of pigment (Fig 2). This may be due to the best utilization of nutrients by the fungus for optimum productivity of pigments. Ten days incubation period led to the highest

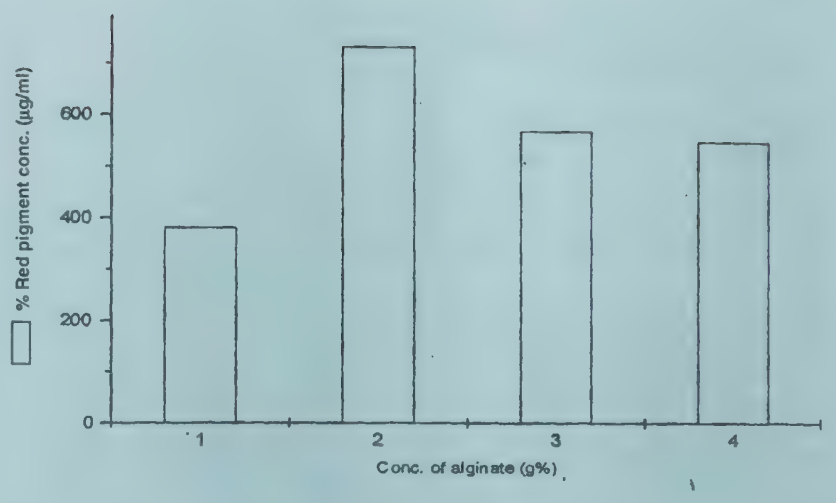

Fig I. Effect of alginate concentration on red pigment production by immobilized *Monascus ruber* ATCC 8111 mycelium.

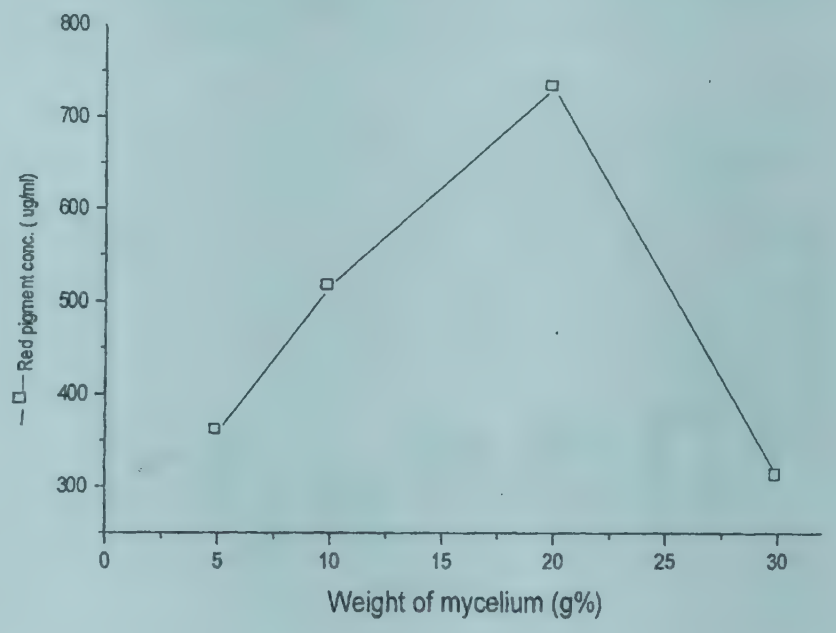

Fig 2. Effect of wet weights of mycelium on red pigment production by immobilized *Monascus ruber* ATCC 8111

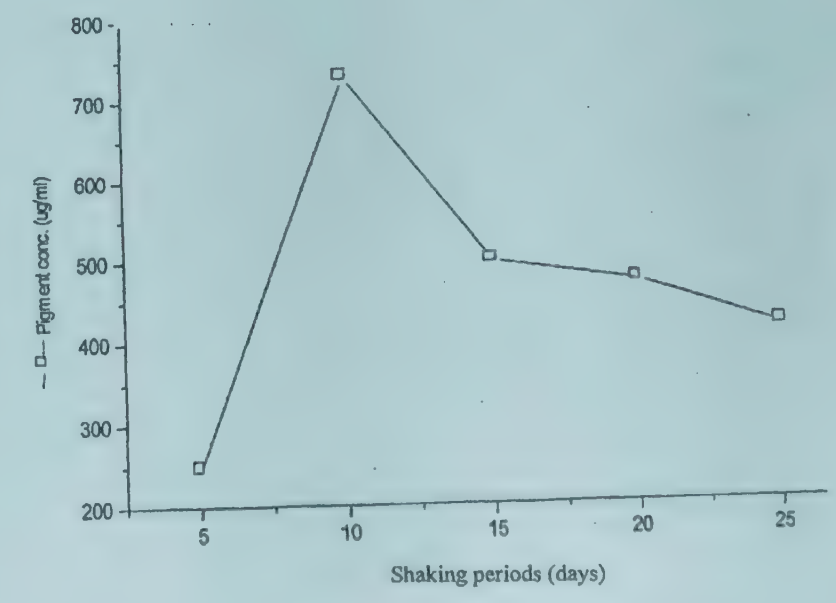

Fig 3. Effect of shaking periods on red pigment production by immobilized *Monascus ruber* ATCC 8111 mycelium.

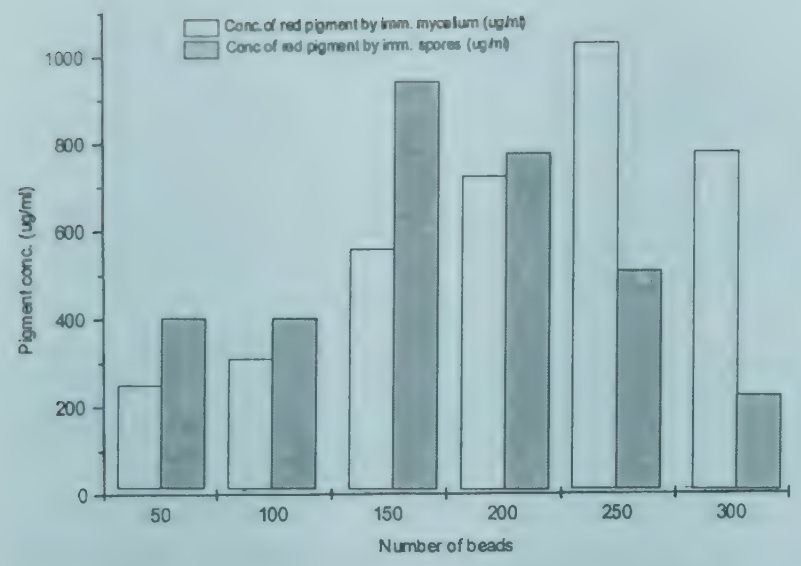

Fig 4. Effect of number of immobilized mycelium and spore beads on red pigment production by *Monascus ruber* ATCC 8111.

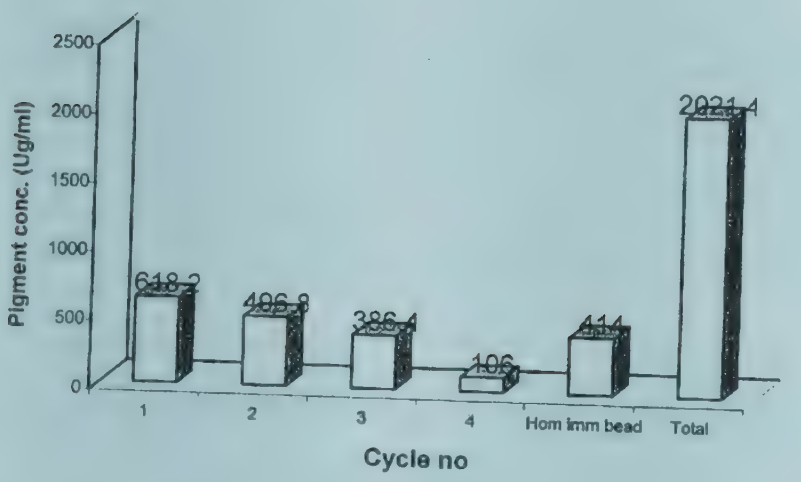

Fig 5. Semicontinuous production of red pigments using immobilized mycelium of *Monascus ruber* ATCC 8111.

Hom. imm. beads : Homogenized immobilized beads
Total : Total produced pigments.

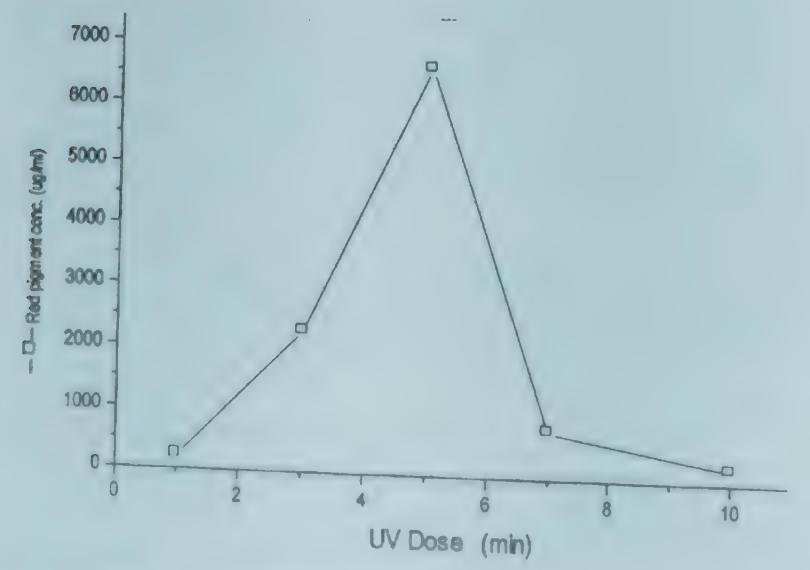

Fig 6. Effect of ultraviolet irradiation on red pigment produced by *Monascus ruber* ATCC 8111.

pigment content (Fig 3). Incubation for 15 and 20 days produced nearly similar and lower contents of pigments. This might be due to the utilization of pigments as an energy source after the depletion of glucose (Turner 1971).

For the highest pigment production, 250 immobilized mycelium beads / 50 ml fermentation medium was the optimum (Fig 4); 300 beads / 50 ml media produced lower yields and this might be due to the competition between cells for nutrients and mineral utilization. Also, decreasing the number of pellets gave smaller output of pigments. This might be due to lower amount of mycelia that led to a reduction in productivity of pigments.

A comparison between immobilized mycelia and immobilized spores (Fig 4). showed the former to be better with regards to the pigments yield.

Semi-continuous production of pigment using immobilized mycelium showed maximal pigment production at the end of the first cycle (Fig 5). Pigment concentration decreased at the end of the other three cycles. This could be attributed to bead disintegration (Abd- El Salam 1996). The net results of pigments obtained by the semicontinuous immobilization by adding to values of the extracted pigments from the filtrate of the four cycles together with that obtained from the mycelial beads was very high as compared with that obtained from the immobilized cells after 10 days.

Experiments with ultraviolet irradiated spores of *Monascus ruber* ATCC 8111 (Fig 6) indicated a 4-fold increase in pigment production at 3 min of exposure to UV irradiation *vis-a-vis* the wild type. A 5 min exposure gave 10 fold increase in productivity compared to the wild type. Condensed doses of UV irradiation for 7 and 10 min sharply reduced pigment output. These results are in accordance with that of many investigators (Lin and Suen 1973; Wong and Koehler 1981). On the other hand, Hiroi et al (1979) obtained hyper productive strains for pigment production by UV irradiation of the wild type for 2.5 min.

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RESEARCH PAPER

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Microbiological Quality of Milk, Vegetables and Fruit Juices

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A total of 36 samples of milk (raw and pasteurized), vegetables and fruit juices were examined for total viable count, staphylococcal count, total coliforms, faecal coliforms, *Salmonella and Shigella*. Total viable count in raw and pasteurized milk was found to be in the range of 15,900 x 10^6 to 2,59,000 x 10^6 and 154 x 10^6 to 24000 x 10^6 cfu/100 ml, respectively. In vegetables and fruit juices TVC ranged from 1690 x 10^6 to 16000 x 10^6 cfu/100 g and 68 x 10^6 to 2080 x 10^6 cfu/100 ml respectively. The mean staphylococcal counts were 470 x 10^6 cfu/100 ml, 138 x 10^6cfu/100 g and 147 x 10^6 cfu/100 ml in raw milk, vegetables and fruit juices respectively. Total coliforms were detected in all the food samples and were found to be in the range of 1600 to >2400 MPN/100 ml, with mean counts of 2.4 x 10^3 MPN/100 ml in raw milk, 2.13 x 10^3 MPN/100 ml in pasteurized milk as well as in vegetables and 2.24 x 10^3 MPN/100 ml in fruit juices. Faecal coliforms in all the samples of raw milk, pasteurized milk, vegetables and fruit juices were found to be in the range of 900-2400, 500-2400, 900-1600 and 900-2400 MPN/100 ml with mean counts as 1.9 x 10^3, 1.5 x 10^3 and 1.36 x 10^3 MPN/100 ml and 1.64 x 10^3 MPN/100 ml respectively. *Salmonella* and *Shigella* were not detected in any of the food samples.

Keywords : Microbiological quality, Milk, Vegetables, Fruit juices, Staphylococci, Coliforms.

In recent years, the increasing consumer awareness has emphasized the need for microbiologically safe food. Since the human food supply consists basically of plants and animals or products derived from them, it is understandable that our food supply can contain microorganisms in interaction with the foods. When the microorganisms involved are pathogenic, their association with our food is critical from a public health point of view. Serious health hazards due to the presence of pathogenic microbes in food can lead to food poisoning outbreaks (Frazier and Westhoff 1997)

In India, the chances of transmission of diseases through milk and milk products are much higher due to unsatisfactory milk hygiene, adulteration practices, poor health condition of animals and ignorance of dairy workers (Sharma and Joshi 1992). Several workers have reported that the contamination of salad, juices, fruits and vegetables is due to unsanitary cultivation and marketing practices (Geldreich and Bordner 1971; Khan et al. 1992; Singh et al. 1995).

Estimation of *Escherichia coli* is an important part of the microbiological analysis of foods and water for quality assurance (ICMSF 1980). Its presence signifies the occurrence of faecal contamination through lack of hygiene and sanitation and/or inadequate exercise of processing controls in food preparation, handling and distribution. It also signifies possible hazard from potentially pathogenic intestinal organisms. Staphylococcal food poisoning is a common foodborne disease that occurs in most countries of the world (Bergdoll 1989), specially in India due to its warm and humid climate (Desai and Kamat 1998). The total number of viable bacteria in food is an adequate index of the bacteriological quality of raw material used, cleanliness in the food processing area in the plant and the storage conditions (Manja and Sankaran 1994).

The detection of pathogenic bacteria in food helps in controlling foodborne infections, the estimation of level of bacterial contamination in food allows assessing the shelf life of food, which is important from the health point of view and the economic point of view (Veerraju and Rangarao 1990). This paper deals the microbial status of milk, vegetables and fruit juices in terms of total viable count (TVC), total coliform (TC), faecal coliform (FC), total staphylococcal count (TSC) and *Salmonella* and *Shigella*.

Materials and Methods

Collection of food samples : Food samples were collected routinely from the local market of Aligarh city. Samples were collected in a sterilized container and transported to the laboratory immediately for initial processing, so the elapsed time between the sample collection and initial processing did not exceed 3 h. The details of the samples are given in Table1.

All the samples were homogeneously mixed before starting the microbiological examination. Different samples of milk, vegetables and fruit juices were emulsified by suspending 10 ml or 10 g samples in 90 ml sterilized normal saline solution (NSS). The whole mixture was shaken well on cyclomixer for atleast 5 min. Serial dilutions of samples were made. Aliquots of the appropriate dilutions were plated on nutrient agar and on different selective media for the isolation of foodborne microorganisms to assay their colony forming ability or to determine the MPN by multiple fermentation tube technique (Desai and Kamat 1998).

TABLE 1. DETAILS OF FOOD SAMPLES

Category	Specific types
Raw milk	Dairy milk (3)*, Can milk (3)
Pasteurized milk	Parag milk (3), Apar milk (3), Rama milk (3)
Vegetables (raw)	Tomato (3), Spinach (3) and radish (3)
Fruit juices	Sugarcane (3), Orange (Tight skinned) (3)
	Pomegranate (3) and Pineapple (3)

*Figures in the parenthesis represent number of samples tested
Parag, Apar and Rama are brand names.

*Corresponding Author

Total viable count : Total viable count of all the food samples were determined by direct plate count as described in standard methods (APHA 1992) using nutrient agar medium. Serial dilutions of the samples were made in NSS as described above; 0.1 ml from each dilution (10^{-2} to 10^{-6}) was plated on nutrient agar medium. The plates were incubated at 37^{0}C for 24 h. After incubation, colonies were counted using colony counter and plates with colonies between 30 and 300 were accounted.

Total Staphylococcal count : The isolation of *Staphylococcus* spp. from food sample was done according to the standard procedure described in APHA (1992). Samples were serially diluted and spread on the plates containing selective media (Staphylococcus agar; HI-media, India) for Staphylococci. The plates were then incubated at 37^{0}C for 24 h. After incubation, the colonies were counted with the help of colony counter. The suspected colonies were picked up, subcultured, and were then finally identified on the basis of morphological, cultural and biochemical characteristics for the isolation of pure culture of Staphylococci (APHA 1992).

Total coliform and faecal coliform : Tests for the presence or absence of TC and FC were done according to the standard methods (APHA 1992). Aliquot of samples (10 ml) were taken in single strength of Mac Conkey's broth and incubated at 37^{0}C for 48 h; 0.1 ml of aliquot from positive samples indicating acid and gas production were transferred to EC broth (HI-Media, India) and incubated at 44.5+0.2^{0}C in a shaking water bath for 24 h. Furthermore, loopfull of culture from the positive tubes was streaked on eosin methylene blue (EMB) agar plates and incubated at 37^{0}C for 24 h. The suspected colonies were picked up, subcultured, and were then finally identified on the basis of morphological, cultural and biochemical characteristics for the isolation of pure culture of *E. coli* (APHA 1992).

Salmonella and shigella : The presence or absence of *Salmonella* and *Shigella* in food samples was detected on Salmonella-Shigella agar (HI-Media, India) and triple sugar iron agar medium as described in APHA (1992).

Results and Discussion

In the present investigation, TVC in raw and pasteurized milk was found to be in the range of 15,900 x 10^6 to 2,59,000 x 10^6 cfu/100 ml and 154 x 10^6 to 24,000 x 10^6 cfu/100 ml respectively (Table 2). Similar findings were also reported by earlier workers (Savitri et al. 1992; Blowey and Collis 1992; Garg and Mandokhot 1997). Garg and Mandokhot (1997) tested 86 raw milk samples for standard plate counts and TVC

TABLE 2. MICROBIOLOGICAL COUNTS IN MILK, VEGETABLES AND FRUIT JUICES

Samples	Minimum counts	Maximum counts	Mean counts	SD	% CV
A. Total viable counts (cfu/100 ml or g)					
Milk (a) Raw	15900 x 10^6 (10.20)*	259000 x 10^6 (11.41)	98500 x 10^6 (10.99)	113483.9 x 10^6	115.2
(b) Pasteurised	154 x 10^6 (8.18)	24000 x 10^6 (10.38)	15000 x 10^6 (10.17)	10624.1 x 10^6	70.8
Vegetables	1690 x 10^6 (9.22)	16000 x 10^6 (10.20)	6580 x 10^6 (9.82)	6662.6 x 10^6	101.3
Fruit Juices	68 x 10^6 (7.50)	2080 x 10^6 (9.32)	723 x 10^6 (8.86)	810.1 x 10^6	112.0
B. Staphylococcal counts (cfu/100 ml or g)					
Milk (a) Raw	70 x 10^6 (7.84)	720 x 10^6 (8.86)	470 x 10^6 (8.67)	287.6 x 10^6	61.2
(b) Pasteurised	ND	ND	ND	ND	ND
Vegetables	78 x 10^6 (7.89)	200 x 10^6 (8.30)	138 x 10^6 (8.14)	49.8 x 10^6	36.1
Fruit Juices	32 x 10^6 (7.83)	520 x 10^6 (8.72)	147 x 10^6 (8.17)	188.1 x 10^6	127.9
C. Total coliform (MPN/100 ml)					
Milk (a) Raw	≥2.4 x 10^3 (3.38)	≥2.4 x 10^3 (3.38)	≥2.4 x 10^3 (3.38)	ND	ND
(b) Pasteurised	1.6 x 10^3 (3.20)	≥2.4 x 10^3 (3.38)	2.13 x 10^3 (3.33)	0.376 x 10^3	17.6
Vegetables	1.6 x 10^3 (3.20)	≥2.4 x 10^3 (3.38)	2.13 x 10^3 (3.33)	0.376 x 10^3	17.6
Fruit Juices	1.6 x 10^3 (3.20)	≥2.4 x 10^3 (3.38)	2.24 x 10^3 (3.35)	0.320 x 10^3	14.3
D. Faecal coliforms (MPN/100 ml)					
Milk (a) Raw	0.9 x 10^3 (2.95)	≥2.4 x 10^3 (3.38)	1.9 x 10^3 (3.28)	0.707 x 10^3	37.2
(b) Pasteurised	0.5 x 10^3 (2.69)	≥2.4 x 10^3 (3.38)	1.5 x 10^3 (3.18)	0.778 x 10^3	51.8
Vegetables	0.9 x 10^3 (2.95)	1.6 x 10^3 (3.20)	1.36 x 10^3 (3.13)	0.330 x 10^3	24.3
Fruit Juices	0.9 x 10^3 (2.95)	≥2.4 x 10^3 (3.38)	1.64 x 10^3 (3.21)	0.671 x 10^3	40.9

Salmonella and *Shigella* were not detected in any of the samples

* Values in parentheses show $\log_{10}$ values

ND Not detected

TABLE 3. MICROBIAL PROFILE OF DIFFERENT CATEGORIES OF MILK, VEGETABLES AND FRUIT JUICES

Source/Type	Mean TVC X 10^6 cfu/100 ml or g	Mean TSC X 10^6 cfu/100 ml or g	Mean TC MPN/100 ml	Mean FC MPN/100 ml	Mean *Salmonella & Shigella* cfu/100 ml or g
Raw milk					
Can milk	139850*	675	≥ 2400	≥ 2400	ND
Dairy milk	15900	70	≥ 2400	900	ND
Pasturized milk					
Parag milk	21100	ND	1600	500	ND
Apar milk	24000	ND	≥ 2400	≥ 2400	ND
Rama milk	154	ND	≥ 2400	1600	ND
Vegetables					
Tomato	1690	200	≥ 2400	1600	ND
Spinach	2050	137	≥ 2400	1600	ND
Radish	16000	68	1600	900	ND
Fruit juices					
Pineapple	1240	97	1600	900	ND
Pomegranate	130	32	≥ 2400	900	ND
Orange(tight skinned)	68	44	≥ 2400	1600	ND
Sugarcane	1088.5	280	≥ 2400	≥ 2400	ND

ND Not detected

* Each value is the mean of 3 replicate samples.

which ranged from 7 x 10^4 to 2 x 10^{10}/ ml. TVC in vegetables and fruit juices ranged from 1,690 x 10^6 to 16,000 x 10^6 cfu/ 100 g and 68 x 10^6 to 2080 x 10^6 cfu/100 ml respectively. (Tables 2 and 3). Garg et al (1993) reported a high range of TVC in different vegetables which ranged from 56 x 10^3 to 52 x 10^8 cfu/g. Singh et al (1995) found that SPC in the range of 2 x 10^6 to 5.5 x 10^6 cfu/ml in orange juice and 1.2 x 10^6 to 2.0 x 10^6 cfu/ml in sugarcane juice. The total staphylococcal count and total coliform were always much less than TVC. This is quite obvious because TVC represents total number of viable cells including total staphylococcal count and total coliforms.

The mean Staphylococcal counts were 470 x 10^6 cfu/ 100 ml, 138 x 10^6 cfu/100 g and 147 x 10^6 cfu/100 ml in raw milk, vegetables and fruit juices respectively (Table 2). Total coliforms were detected in all the food samples and were found to be in the range of 1600 to ≥ 2400 MPN/100 ml, with mean counts as high as 2.4 x 10^3 MPN/100 ml in raw milk, 2.13 x 10^3 MPN/100 ml in pasteurized milk as well as in vegetables and 2.24 x 10^3 MPN/100 ml in fruit juices. Faecal coliforms in all the samples of raw milk, pasteurized milk, vegetables and fruit juices were found to be in the range of 900-2400, 500-2400, 900-1600 and 900-2400 MPN/100 ml, with mean counts as 1.9 x 10^3, 1.5 x 10^3, 1.36 x 10^3 and 1.64 x 10^3 MPN/100 ml respectively (Table 2). However, *Salmonella and Shigella* were not detected in any of the food samples tested. These findings are similar with the earlier reports (Carmo et al. 1990; Tchango et al. 1992; Ombui et al. 1994; Srihari and Rao 1998). Abbar and Kaddar (1991) tested 400 samples of Iraqi milk products and found faecal coliforms at levels higher than 10^2 cfu/g in 72.5% and < 10^2 cfu/g in 27.5% of samples. Srihari and Rao (1998) tested milk samples for the presence of *E. coli* using different media. *E. coli* was detected in 20% of the samples and the most probable numbers were estimated to be 2.5 X 10^2 and 2.0 X 10^2 cfu/ml respectively in EC broth and EC broth with 4- methyl umbelliferyl β-D-glucuronide (MUG) simultaneously. Soto and Rodriguez (1988) examined 10 vegetables samples each of tomato, endine, pepper and carrot or 5 samples each of onion and cucumber and reported that 14.0% samples contained *E. coli*, 13.1% *S.aureus* and 0.5% *Ps. aeruginosa*. Anand and Singh (1989) performed microbiological analysis of 102 infant milk food samples and found that 22.5% of the samples were positive for coliforms, 57.8% of the samples were positive for Staphylococci and about 80% of the samples were well within the standard limits of total viable count as per the Indian Standard Institution. *Salmonella* and *Clostridia* were not found in any of the samples by these authors. Hildebrandt et al (1989) examined the hygienic quality of salads made from raw vegetables and found that all the samples were free from

S. aureus and *E. coli*. Tchango et al (1992) performed microbiological determinations on pineapple juice, guava, passion fruit and grape fruit nectars in Cameroon and found *E. coli* in pineapple juice only.

Salad vegetables are often consumed fresh and thus can act as effective media for the transmission of associated pathogens. Khan et al. (1992) found 7.1 x 10^4 to 6.34 x 10^8 cfu/100 g bacterial load in fresh samples of salad vegetables like cucumber, carrot and lettuce in Bangladesh (Dhaka). Sharma and Joshi (1992) found no Salmonellae in 69 milk samples and they thought that *Salmonella* in milk might escape detection because of their usually small number, or the growth of *Salmonella* might have been inhibited by some other fast growing organisms. Garg et al (1993) found coliforms in the range of $\leq 10^1$ to 48 x 10^3 counts/g in different vegetables. Ombui et al (1994) tested raw milk in Kenya and found that 42.2% of the milk samples collected from farmers cans and 10.3% samples collected from cooperative cans were found to be free of coliforms while 89.5% of the samples collected from farmers cans and 50% samples collected from cooperative cans were considered to be of good quality with no more than 50,000 coliforms/ml of milk. Singh et al (1995) reported 4 x 10^5-3 x 10^6 coliform count/100 ml in orange juice and 2.1 x 10^6 - 2.3 x 10^6 coliform count/100 ml in sugarcane juice.

In conclusion, it may be suggested that microbiological quality of milk (pasteurized and unpasteurized), vegetables and fruit juices has been observed to be poor especially in view of the higher levels of coliforms and Staphylococcal counts as compared to the maximum permissible limits for raw food consumption (ICMSF 1998).

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RESEARCH PAPER

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Coating of Cashew Kernel Baby Bits for Value Addition

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Value addition of cashew (*Anacardium occidentale* L.) kernel baby bits (CKBB) has been attempted by coating with cane sugar, honey and salt. Optimum coating occurs at 100°C for 5 min at 70% concentration for cane sugar and honey and 5% for salt. Sweetened (70%) and vanillin (0.1%) flavoured CKBB are the most preferred. Defatting of CKBB enhances the per cent coating. Coating of cashew kernels of different grades with cane sugar at 70% is dependent on the surface area. Cashew apple juice could be coated on the CKBB. Acceptability of cashew apple juice coated baby bits (BB) improves with the addition of cane sugar at 70% concentration. Permitted colours and cane sugar compete with each other during coating.

Keywords : *Anacardium occidentale* L., Cashew, Coating, Cane sugar, Flavour, Honey, Permitted colours, Salt, Value addition.

Commercial processing of cashew nuts involves number of steps like, roasting, shelling, peeling and packing. Cashew processing results in recovery of kernels of different grades such as wholes, splits, bits etc. Value addition in cashew is one of the emerging areas. Earlier, attempts have been made for coating of cashew kernels with salt (Jasper Guy Woodruff, 1971; Prasad, 1954; Prasad et al. 1955; Vidyasagar et al. 1966) and preparation of burfi (Satyanarayana Rao et al., 1993). Cashew kernel wholes fetch maximum price while cashew kernel baby bits (CKBB), the lowest grade kernels obtained during processing of cashew nuts, fetch less. About 0.5 to 1% of CKBB are obtained during commercial processing. These are sold at Rs. 120 to 140/kg in the local market. The present investigation has been undertaken to develop value added products without introducing external oil by coating CKBB with cane sugar, honey, salt and cashew apple juice.

Materials and Methods

Cashew kernel baby bits (CKBB) and almond were purchased from M/s Achal Industries, Mangalore. Cane sugar, honey, iodised salt, groundnut and permitted colours were purchased from the local market. All the other chemicals used were of analytical reagent grade. Cardamom capsules were procured from Cardamom Research Centre, Indian Institute of Spices Research, Appangala, Kodagu and essential oil was recovered by hydro distillation and used for coating studies. The samples were thoroughly washed with distilled water to remove adhering testa particles and dried at 70°C for 6 h. These were defatted with n-hexane at ambient temperature (27°C to 30°C) over a period of 48 to 72 h with frequent changing of solvent and dried at 70°C for 6 h.

Coating of CKBB with cane sugar, honey and salt was carried out at different temperatures and concentrations for 5 min and were dried at 70°C for 3 to 4 h. Ratio of CKBB to the coating solution was 1:2 (5 g/10 ml). Similarly, coating of CKBB with clarified whole cashew apple juice, aroma stripped cashew apple juice and aroma distillate was carried out. Freshly harvested and washed cashew apples were squeezed and the recovered juice was boiled for 15 min in presence of 0.05% gelatin and allowed to settle. Aroma from the clarified juice was recovered by steam distillation.

Coated baby bits (1 g) (BB) were extracted with distilled water at 10°C for 12 h and sugar was estimated for assessing the extent of coating (Dubois et al. 1951). Similarly extent of coating of salt was assessed by measuring the conductivity of the extract. Esters in the extract of cashew apple juice coated baby bits were estimated using ethyl acetate as standard (Leonard and Willard 1960).

Coated CKBB were assessed by a panel of judges using a 9-point Hedonic scale for colour, flavour, texture and taste. Mean cumulative scores for these characters were calculated for comparison of different coated CKBB.

Results and Discussion

Cashew kernel baby bits (CKBB) were coated with cane sugar, honey and iodised salt at temperatures varying between 60 and 100°C at different concentrations for 5 min (Fig. 1 to 3). In the case of honey, coating at concentrations beyond 70% could not be tried as the solution became very viscous. Per cent coating of cane sugar increased with increased

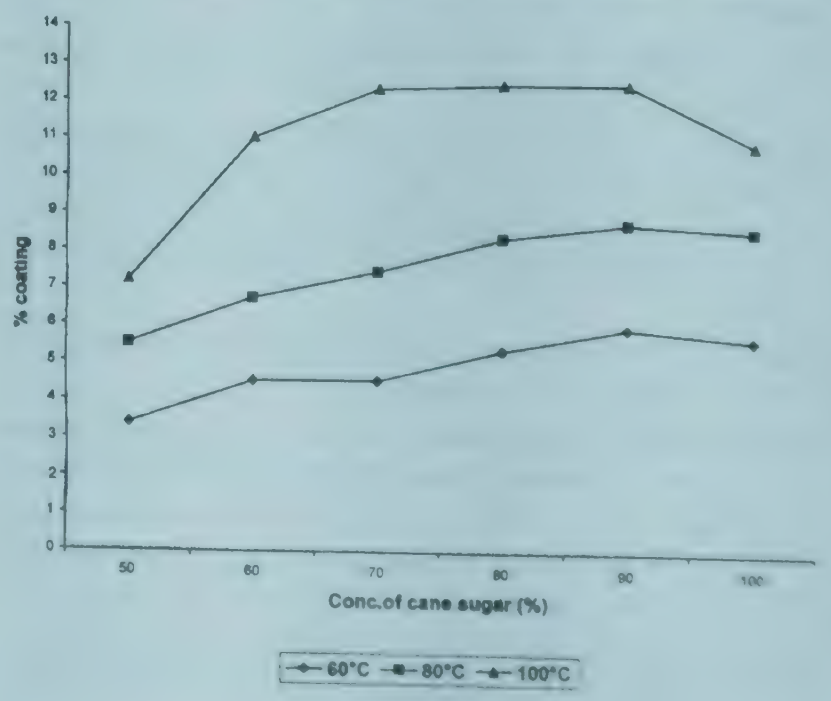

Fig. 1. Coating of CKBB with cane sugar at different temperatures

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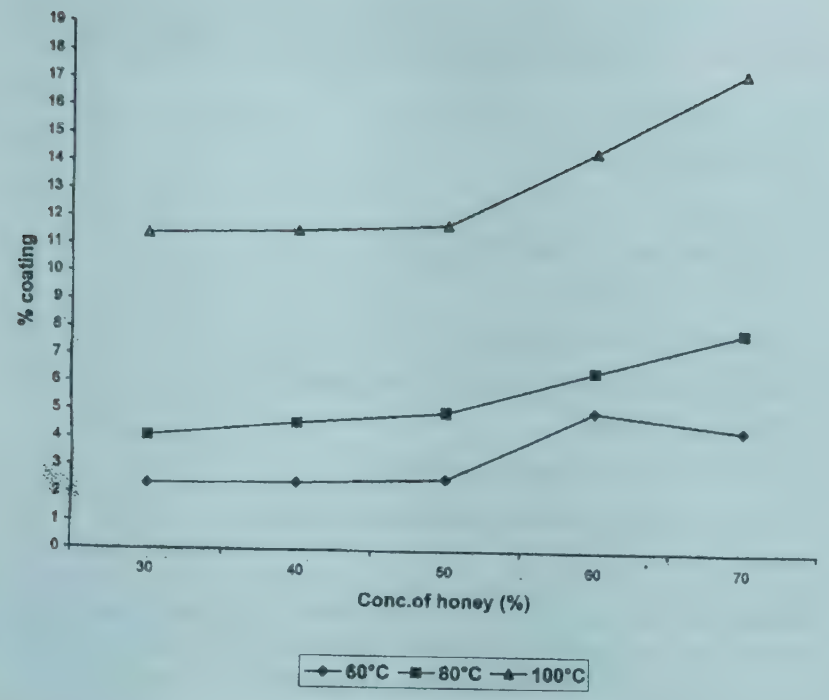

Fig. 2. Coating of CKBB with honey at different temperatures

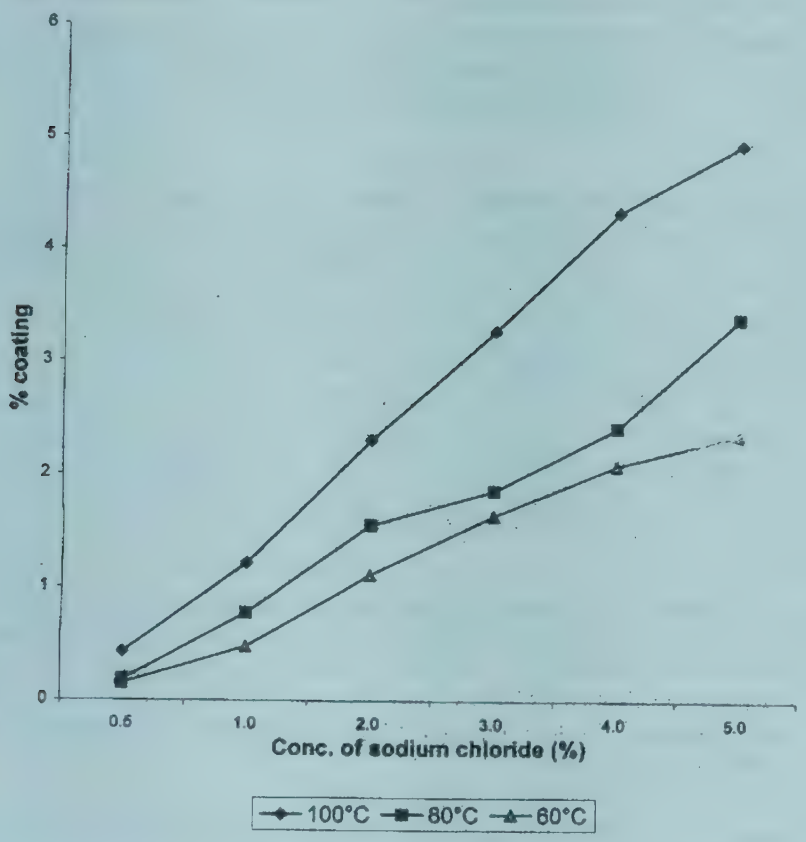

Fig. 3. Coating of CKBB with sodium chloride at different temperatures

temperature and concentration. Coating beyond 70% concentration did not differ much. Optimum temperature for coating of BB with cane sugar, honey and iodised salt was 100°C. Optimum concentration for coating of BB with cane sugar and honey was 70%. Similarly, optimum concentration for coating of baby bits with salt was 5%.

CKBB, almond and ground nut (after removing the testa) were defatted and were coated with cane sugar and honey at 70% concentration at 100°C for 5 min (Table 1). Defatting of kernels enhances the extent of coating of honey and cane sugar.

Coating of whole and defatted CKBB at different temperatures with cane sugar and honey was compared.

TABLE 1. COATING OF DEFATTED KERNELS WITH CANE SUGAR AND HONEY.

	% Coating			
	Cane sugar		Honey	
	Control	Defatted	Control	Defatted
Cashew kernel baby bits	17.0	20.13	13.23	22.89
Groundnut halves	8.38	6.88	6.88	9.87
Almond halves	5.1	8.60	2.77	10.78

Values are mean of 3 individual estimations. Coating was done at 100°C for 5 min. at 70% concentration. Coated kernels were extracted for 12 h at 10°C for estimation of sugars.

	Sugar	Honey
CD (p=0.01) for % coating of whole and defatted kernels	4.13	6.62
CD (p=0.01) for % coating of different kernels	3.9	2.79

TABLE 2. EFFECT OF DEFATTING ON PER CENT COATING OF CKBB WITH CANE SUGAR AND HONEY AT DIFFERENT TEMPERATURES

Temperature (°C)	% Coating			
	Cane sugar		Honey	
	Control	Defatted	Control	Defatted
100	9.70	16.10	13.90	22.89
80	4.45	9.36	8.67	15.33
60	5.71	12.20	6.36	13.89

Values are mean of 3 individual estimations. Coating was done at 100°C for 5 min. at 70% concentration. Coated kernels were extracted for 12 h at 10°C for estimation of sugars.

	Sugar	Honey
CD (p=0.01) for % coating of baby bits at different temperatures	5.05	3.35
CD (p=0.01) for % coating of whole and defatted baby bits	5.04	6.04

Enhanced coating of defatted CKBB was noticed at all the temperatures (Table 2).

Cashew kernels of different grades were coated with 70% cane sugar for 5 min at 100°C (Table 3). Per cent coating is directly proportional to the counts / 5g and inversely proportional to the weight/kernel. Higher coating of cane sugar is observed in BB (18.6%) compared to W 150 kernels (1.69%). Thus per cent coating is dependent on the surface area, and CKBB offer more surface area for coating.

CKBB were coated with whole clarified cashew apple juice, aroma stripped cashew apple juice and aroma distillate in presence of 70% cane sugar at different temperatures (Table 4). Irrespective of temperature of coating, inclusion of 70% cane sugar enhanced the extent of coating. Aroma distillate could be coated in presence of cane sugar to impart cashew apple flavour to the coated BB. Cashew apple juice coated BB were extracted and esters were estimated in the extracts (Table 5). Coating of esters gets reduced in presence

TABLE 3. COATING OF CASHEW KERNELS OF DIFFERENT GRADES WITH CANE SUGAR

Grade	Counts/5 g	Weight/kernel (mg)	% coating
BA	550.00	9.09	18.60
BBA	250.00	20.00	15.12
SDP	90.00	55.50	11.00
SWP	55.00	90.90	10.06
W 450	4.60	1086.90	7.88
W 320	3.46	1445.00	4.38
W 240	2.60	1923.00	4.17
W 210	2.13	2347.00	2.57
W 180	1.78	2808.00	2.93
W 150	1.44	3472.00	1.69
		CD (p=0.01)	2.79

Values are mean of 3 individual estimations. Coating was done at 100°C for 5 min. at 70% concentration. Coated kernels were extracted for 12 h at 10°C for estimation of sugars.

TABLE 4. COATING OF CKBB WITH CASHEW APPLE JUICE AT DIFFERENT TEMPERATURES (°C)

Coating agent	% Coating at 60°C	80°C	100°C
Clarified cashew apple juice	3.75	4.98	10.34
Clarified cashew apple juice containing 70% cane sugar	14.97	10.92	15.32
Aroma stripped clarified cashew apple juice	2.43	5.67	8.02
Aroma stripped clarified cashew apple juice containing 70% cane sugar	8.57	13.19	15.24
Cashew apple aroma distillate containing 70% cane sugar	9.15	13.31	17.19
70% cane sugar	10.19	10.82	17.55

Values are mean of 3 individual estimations. Coating was done at 100°C for 5 min. at 70% concentration. Coated kernels were extracted for 12 h at 10°C for estimation of sugars.

CD (p=0.01) for different coating agents = 1.6

CD (p=0.01) for % coating at different temperatures = 1.48

of 70% cane sugar. Maximum per cent coating of esters was observed in the extract of 70% honey coated BB. Least per cent coating of esters was noticed with cardamom essential oil coated BB.

CKBB were coated at different concentrations of cane sugar and honey for 5 min at 100°C and were assessed for colour, flavour, texture and taste by a panel of 20 judges (Table 6). Although maximum coating of both cane sugar and honey occurs at 70% concentration, CKBB coated with 50% cane

TABLE 5. ESTIMATION OF ESTERS IN COATED CKBB

Coating agent	% coating
70% cane sugar	Zero
70% honey	4.18
70% cane sugar containing 0.2% cardamom essential oil	0.019
Clarified whole cashew apple juice	1.51
Clarified whole cashew apple juice containing 70% cane sugar	0.53
Aroma stripped cashew apple juice	0.79
Aroma stripped cashew apple juice containing 70% cane sugar	0.42

Values are mean of 3 individual estimations. Coating was done at 100°C for 5 min. at 70% concentration. Coated kernels were extracted for 12 h at 10°C for estimation of esters.

TABLE 6. SENSORY EVALUATION OF CKBB COATED WITH CANE SUGAR AND HONEY AT DIFFERENT CONCENTRATIONS.

Concn. (%)	Cane sugar		Honey	
	% coating	Mean cum. Hedonic score	% coating	Mean cum. Hedonic score
30	2.67	13.12	3.56	12.82
40	5.26	12.93	5.29	13.41
50	9.33	12.12	8.39	14.29
60	10.81	12.19	8.22	13.12
70	13.03	13.24	13.80	16.12
CD at p = 0.01		1.68	2.83	2.03

Mean cumulative Hedonic scores represent the cumulative Hedonic score for colour, flavour, texture and taste of the coated kernel baby bits of 20 judges. Values are mean of 3 individual estimations. Coating was done at 100°C for 5 min. at 70% concentration. Coated kernels were extracted for 12 h at 10°C for estimation of sugars.

sugar and 30% honey are the most preferred with mean cumulative Hedonic score of 12.12 and 12.82, respectively. Similarly, CKBB were coated at 100°C with different concentrations of salt and were assessed for colour, flavour, texture and taste by a panel of 20 judges. Although maximum coating of salt was noticed at 5%, CKBB coated with 0.5% iodised salt was the most preferred with mean cumulative Hedonic score of 14.26 (Table 7).

CKBB were coated individually with different sugars, cane sugar containing different flavours and cashew apple juice and were assessed for sensory characters by a panel of 20 judges (Table 8). Sweetened (70% cane sugar) and vanillin (0.1%) flavoured BB were the most preferred with a mean cumulative Hedonic score of 10.84. Inclusion of cane sugar in either clarified or whole cashew apple juice improved the acceptability of coated BB with reduced mean cumulative hedonic score

TABLE 7. SENSORY EVALUATION OF CKBB COATED WITH SALT AT DIFFERENT CONCENTRATION.

Concentration (%)	% coating	Mean cum. Hedonic score
0.5	0.153	14.26
1.0	0.884	14.63
2.0	1.88	14.68
3.0	3.14	15.53
4.0	3.56	15.63
5.0	4.73	16.63
CD (p=0.01)	0.203	2.06

Mean cumulative Hedonic scores represent the cumulative Hedonic score for colour, flavour, texture and taste of the coated kernel baby bits of 20 judges. Values are mean of 3 individual estimations. Coating was done at 100°C for 5 min. at 70% concentration. Coated kernels were extracted for 12 h at 10°C for estimation of salt.

CKBB coated with cashew apple aroma containing 70% cane sugar was preferred (13.21) over those coated with cashew apple aroma alone (14.85). Acceptability of CKBB coated with clarified cashew apple juice was comparable with uncoated CKBB.

CKBB were coated at 100°C for 5 min. with different colours at 0.1% concentration dissolved either in distilled water or 70% cane sugar solution. Coated BB were extracted for 12 h at 10°C. Concentration of colour and sugar in the extract were determined (Table 9). Per cent coating of colours gets reduced in presence of cane sugar. Similarly extent of sugar coating gets affected in presence of colours. CKBB were coated with apple green and raspberry red in presence of varying concentrations of cane sugar to assess the effect of

TABLE 8. SENSORY EVALUATION OF CASHEW KERNEL BABY BITS COATED WITH DIFFERENT COATING AGENTS

Coating agent	% coating	Mean cum. Hedonic score
Nil (uncoated baby bits)	--	14.85
70% Glucose	13.42	13.37
70% Fructose	12.45	12.56
70% Sucrose	13.38	13.06
70% Lactose	13.38	16.50
70% Honey	17.28	16.52
70% Cane sugar	11.14	13.84
70% Cane sugar with 0.1% vanillin	9.69	10.84
70% Cane sugar with 0.2% essential oil of cardamom	11.09	12.21
Clarified cashew apple juice	5.94	14.58
Clarified aroma stripped cashew apple juice	3.87	17.00
Clarified cashew apple juice containing 70% cane sugar	7.36	12.58
Clarified aroma stripped cashew apple juice containing 70% cane sugar	10.19	11.95
Cashew apple aroma distillate	--	14.85
Cashew apple aroma distillate containing 70% cane sugar	12.94	13.21
CD (p=0.01)		2.57

Mean cumulative Hedonic scores represent the cumulative Hedonic score for colour, flavour, texture and taste of the coated kernel baby bits of 20 judges. Values are mean of 3 individual estimations. Coating was done at 100°C for 5 min. at 70% concentration. Coated kernels were extracted for 12 h at 10°C for estimation of sugars.

TABLE 9. COATING OF CKBB WITH DIFFERENT COLOURS IN DISTILLED WATER AND 70% CANE SUGAR SOLUTION.

Colour	λ max (nm) (aq. solution)	% coating Colour		Cane sugar
		Water	70% cane sugar	70% cane sugar
Apple green (IH 8925)	430	0.155	0.065	19.26
Chocolate brown (IH 8962)	430	0.075	0.041	28.52
Kesari powder (IH 9140)	430	0.068	0.036	17.66
Lemon yellow (IH 6597)	430	0.064	0.047	25.42
Orange red (IH 7802)	500	0.092	0.023	17.14
Raspberry red (IH 7804)	515	0.04	0.016	13.67
70% cane sugar (control)	–	–	–	34.08

BB were coated at 100°C for 5 min. Ratio of BB to coating solution was 1:2 (5 g / 10 ml). For coating, colours at 0.1% concentration was dissolved either in distilled water or 70% cane sugar solution. Coated BB were extracted at 10°C for 12 h and absorbance was read after centrifugation for determining the % coating of colour. Similarly % coating of cane sugar was assessed by determining the total sugar in the extracts of coated baby bits. Values are the mean of 4 individual estimations.

CD (p=0.01) for % coating of colour with and without 70% cane sugar = 0.01

CD (p=0.01) for % coating of different colours with and without 70% cane sugar = 0.0066

CD (p=0.01) for % coating of cane sugar with and without colour = 6.00

TABLE 10. COATING OF CKBB WITH COLOURS AT VARYING CONCENTRATION OF CANE SUGAR.

Cane sugar concentration (%)	% Coating			
	Apple green		Raspberry Red	
	Colour	Sugar	Colour	Sugar
0	0.09	-	0.040	-
30	0.053	10.10	0.029	6.04
40	0.043	11.45	0.030	9.82
50	0.037	11.75	0.025	11.78
60	0.032	13.74	0.021	11.92
70	0.034	14.34	0.017	12.37
CD (p=0.01)	0.007	2.27	0.0076	2.36

CKBB were coated at 100^{0}C for 5 min with 0.1% concentration of colour at varying concentrations of cane sugar. Other conditions are same as in Table 9. Values are mean of 6 individual estimations.

cane sugar concentration on coating of colour (Table 10). Per cent coating of colour is inversely proportional to the concentration of cane sugar. Optimum coating of colour occurs in the absence of cane sugar. Thus, both colour and the sugar compete with each other during coating.

Conclusion

Optimum coating of CKBB occurs at 100^{0}C for 5 min at 70% concentration for cane sugar and honey and 5% for salt. CKBB coated with 50% cane sugar, 30% honey and 0.5% salt are preferred when assessed for acceptability. CKBB could be coated with clarified cashew apple juice whose acceptability could be improved with the inclusion of 70% cane sugar. Sweetened and vanillin flavoured cashew kernel bits are the most preferred. Defatting improves the extent of coating of CKBB with cane sugar, honey and salt. Extent of coating of cane sugar is dependent on the surface area. Permitted colours and cane sugar compete with each other during coating of CKBB.

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RESEARCH PAPER

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Bacteriological Examination of Pasteurized Milk and Milk Products Sold in Harare, Zimbabwe

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A study was conducted to assess the bacteriological quality of pasteurized milk and ice cream sold in Harare, using the direct plate count method and the methylene blue dye reduction test. Altogether, 105 milk and 95 ice cream samples comprising of 46 vanilla and 49 strawberry flavours obtained from two factories (depots) and 5 distributing supermarkets (outlets). Under the methylene blue reduction test, all milk and ice cream samples passed the 30 min and 2 h tests, respectively. However, 99% of the milk and 69% of the ice cream samples reduced the dye after 5 ½ and 4 h, respectively. The results from the direct plate counts revealed the presence of both pathogens and non - pathogens. The median plate counts in the milk and ice cream were found to be 400 and 100 cfu/ml, respectively. Organisms isolated in both samples and in all outlets were similar, these included *Bacillus* spp., coagulase *Staphylococcus* spp., *Micrococcus* spp., *Streptococcus* spp., *Diphtheroids, Fusiform* bacteria, *Klebsiella* spp. and *Citrobacter* spp. No significant differences were found in the plate counts of the samples obtained from the depots and the outlets for the milk ($p=0.542$, $df=1$) and ice cream samples($p=0.377$, $df=1$). Results further revealed that there was no significant difference in isolates obtained from milk ($p=0.803$, $df=5$) and vanilla ice cream ($p=0.279$, $df=5$) using the plate count method. There was, however, significant difference in plate count of isolates obtained from strawberry ice cream (0.0096). The study, revealed that milk and milk products sold in various outlets in Harare may contain variety of bacteria of public health importance and also shown that the methylene blue reduction test is not reliable for the detection of bacterial contaminants in dairy products. The study suggested the use of methylene blue dye in combination with other tests such as the plate count in assessing bacterial contaminants in milk products.

Milk and milk products constitute important nutritional components for all age groups. Various milk products are available in local markets, these products include cheese, fermented milk, ice cream, butter, pasteurized concentrated and dried milk, ultra heat treated dairy products and various desserts. Milk and milk products also provide ideal growth environment for various bacteria (Bryan 1983). Industrialization of milk processes invariably creates potential points of contamination. Pasteurization, which destroys vegetative forms of pathogenic organisms, is employed to check contamination of milk products from industrial production lines. Despite this precaution, coupled with increased level of hygiene, contamination of milk and ice cream has been known to occur.

Different organisms have been incriminated by various workers for their role in contamination of milk and milk products. Gill et al (1994) reported *Staphylococcus aureus* food poisoning, which resulted in milk borne disease. *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* have also been isolated from milk (Wouafo et al. 1996). *Streptococcus* and *Salmonella* species have similarly been associated with contamination of milk products (Gill et al. 1994; Gazzar and North 1992).

Suitable temperatures for bacterial growth are found in retail shops and refrigerators where milk may remain unsold for up to 48 h enabling organisms present to reach high concentration (Omubi et al. 1992). Organisms like *S.aureus* may survive pasteurization, while contaminated water used in the factory and low hygienic standard may predispose to contamination by *Salmonella* species. A multistate *Salmonella* outbreak, which occurred in 1994 in USA was associated with contaminated product lots (Vought and Tatini 1998). *Escherichia coli* is another frequently isolated organism from milk and ice cream. *E.coli* 0157 phage type 2 was implicated in a diarrhoea outbreak traced to pipes carrying pasteurized milk prior to bottling and also to discarded bottling machine rubber (Upton and Coia 1994). There are thus incontrovertible evidences that milk products pose potential health hazards to consumers, if contaminated. Regular examination of these products is, therefore, necessary to assess the extent of microbial contamination, which may pose a public health threat. Manufacturers of milk and its products are usually concerned with contaminants at production point, while ignoring the fate of the products after leaving the factory.

This study was aimed at assessing the bacteriological quality of pasteurized milk and ice cream samples sold to consumers in Harare, Zimbabwe; and was expected to give an insight on the public health implications of consumption of these products from retail outlets.

Materials and Methods

Source of specimens : This study was carried out at the University of Zimbabwe, Department of Microbiology. Sources of specimen were 2 brands of pasteurized milk and 2 brands of ice cream. These products are made by Dorking Dairies (DD) and Dairiboard (DB) Zimbabwe Limited respectively, all situated in Zimbabwe. Milk and ice cream samples were purchased directly from the factories and from five outlet shops (supermarkets) within Harare. During sampling, only one specimen was collected at a time from the factory. The dates

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of manufacture and expiration were noted. Milk and ice cream with same manufacture and expiration dates were traced to outlet shops and samples collected every 2 days while stock lasted. All samples were transported in an insulated container and on arrival to the laboratory samples were examined using standard laboratory procedures as described by Delsot (1977).

Treatments of samples : The sample containers were cleaned using a disinfectant and examined for evidence of leakage or defects in packaging that could introduce contamination. Contents of intact packets only were used for this test. Milk packets were shaken up and down 12 times and inverted 12 times to obtain a homogenous solution (Lyne and Collins 1970). The packets were then opened and the milk poured into sterile 20 ml vials.

Methylene blue reduction test for milk : Methylene blue was prepared using the method described by Kiss (1984). The methylene blue reduction test of milk was performed as described by Cappuccino et al (1996). Briefly, three sterile test tubes were labeled 1-3 and to each tube 10 ml pasteurized milk was added. A 1 ml amount of methylene blue was added to tubes 1 and 2, and the tubes were stoppered and gently inverted for about 4 times. To the 3rd test tube, 1 ml tap water was added and mixed gently with the milk. Tube 2 was placed in a water bath set at 100^0C for 3 min. All the test tubes were then transferred into a second water bath set at 37^0C. After half an hour interval; the tubes were removed from the water bath and examined for any colour change. The dye was considered to have been reduced, if the fluid in the test tubes I and 2 changed from blue to white. Provisional grades were awarded as described by Standard Operating Procedure Manual from Dairiboard, Zimbabwe Limited (Bulawayo) as shown below.

Time for reduction of colour	Class	Remarks
0 - ½ h	4	very unsatisfactory
½ - 2 h	3	unsatisfactory
2 - 5½ h	2	less satisfactory
> 5½ h	1	satisfactory

According to the above Operating standard, all milk samples reducing the dye within 30 min should be subjected to standard laboratory tests to identify the presence of any bacteria.

Direct plating of milk samples : A ten fold serial dilution of milk samples was prepared using quarter strength Ringers solution as described (Lyne and Collins 1970). One milliter of the 10^{-2} dilution was transferred to each of 2 sterile petri dishes. Ten to 12 ml of previously prepared molten agar (plate count agar) was poured over the petri dishes and the contents immediately mixed (using 6 clockwise movements with a diameter of about 150 mm, followed by 6 anti clockwise movements) and finally were then allowed to set, inverted and incubated at 37^0C for 48 h.

Identification and characterization of organisms : All samples were plated in duplicate and subsequent plate counts were conducted every other day. After incubation, the average number of organisms per ml was calculated from the dilution factor formula (Robert et al. 1995). Colony growth on the plate count agar was sub cultured onto blood agar (BA) and MacConkey agar (MCA) plates. Plates were incubated at 37^0C for 24 h. Organisms were characterized using standard laboratory tests.

Methylene blue reduction test for ice cream : Ice cream samples were first placed at refrigerator temperature of 5^0C to melt and then placed at 37^0C for 1 h to liquefy completely before testing. Three sterile test tubes were labeled 1-3. Seven ml quarter strength Ringers solution and 1 ml of methylene blue dye was added to tubes 1 and 2. To tube 3, 8 ml quarter strength Ringers solution was added. Two milliters of ice cream was then added to each of the test tubes. Test tube 2 was placed in a water bath set at 70^0C for 10 min (this tube served as positive control for the methylene blue reduction test). All tubes were then placed in a second water bath set at 20^0C for 18 h. (for possible growth of mesophilic organisms that might be present in the samples) tubes were examined for colour change. The tubes were then transferred to another water bath set at 37^0C (optimum temperature for growth). Ice cream samples showing colour change were observed in a similar way as the milk samples. Provisional grades were also given as described (Lyne and Collins 1970).

Provisional grades		Methylene blue dye
Satisfactory	1	dye not reduced at 4 h
Fairly satisfactory	2	decolourized between 2½ - 4 h
Unsatisfactory	3	decolourized between ½ - 2 h
Very unsatisfactory	4	decolurized before ½ h

Ice cream samples that reduced the dye within the test period (5 ½ h) were subjected to laboratory tests to isolate and identify the contaminants as described earlier for milk samples.

Statistical analysis : Statistical tests using the Kruskal-wallis one way Anova (Kirkwood 1988) were performed to access if there were any significant difference between samples from the various outlets.

TABLE 1. METHYLENE BLUE REDUCTION TEST OF MILK AND ICE CREAM SAMPLES

Time reduction of dye, h	Number of samples reducing the dye		Remarks
	Milk samples	Ice cream samples	
0 - ½	0	0	Very unsatisfactory
½ - 2	1	0	Unsatisfactory
2 - 4	NA	9	Less satisfactory
> 4	NA	20	Satisfactory
2 - 5½	0	NA	Less satisfactory
> 5½	69	NA	Satisfactory
Total	70	29	

NA= not applicable

TABLE 2. QUANTATIVE BACTERIOLOGICAL ANALYSIS OF MILK AND ICE CREAM SAMPLES

Organisms isolated	Number of isolates/ Percentage of number sampled				
	DB Vanilla	Strawberry	Milk	DD milk	Total isolates
Coagulase negative *Staphylococcus* spp.	2 (4.3)	–	20 (36.4)	7 (14)	29
Bacillus spp.	29 (63)	26 (53.1)	18 (32,7)	10 (20)	83
Micrococcus spp.	8 (17.4)	2 (4.1)	8 (14.5)	15 (15)	33
Fusiform bacteria	7 (15.2)	1 (2.0)	9 (16.4)	–	17
Group D *Streptococcus* spp.	6 (13.1)	2 (4.1)	5 (9.1)	10 (20)	23
Non Group D *Streptococcus* spp.	6 (13.0)	3 (6.1)	4 (7.3)	6 (12)	19
Klebsiella spp.	–	–	2 (3.6)	–	2
Citrobacter spp.	–	–	1 (1.8)	–	1
Diptheroids	–	–	4 (7.3)	–	1
Total Isolates	58	34	71	48	
No. of samples screened	46	49	55	50	

DB and DD = Factories codes

Results and Discussion

A total of 105 milk and 95 ice cream samples sold in various outlets in Harare, were examined for bacterial contamination using the Methylene blue reduction test and Plate count methods. The results of the methylene blue reduction tests are shown in Table 1. Of the 105 milk samples examined, none decolourized the blue dye within the first 30 min. However, one milk sample showed colour change after 2 h, while 69 milk samples showed colour change after 5½ h. Altogether 70 (66.6%) of the milk samples showed reduction of methylene blue dye after 30 min. Also a total of 29 ice cream samples reduced the dye, 9 samples decolourized the blue dye between 2-4 h and 20 samples after 4 h.

When milk and ice cream samples were plated directly onto plate count agar and incubated for 48 h; results showed a variety of colony growths of organisms. Characterization and identification of organisms showed them to be coagulase negative *Staphylococcus* spp., *Streptococcus* spp., *Klebsiella* spp., *Citrobacter* spp. and *Diptheroids* (Table 2). The organisms obtained from both milk and ice cream products were similar, but differed in bacterial concentration. A total of 58 isolates were obtained from 46 vanilla ice cream samples. Most prevalent organism was *Bacillus* species (63%). In strawberry ice cream samples, 34 isolates of bacteria were identified from a total of 49 samples with *Bacillus* accounting for 53%.

Fifty samples were collected from Dorking Dairies milk factory, of these 48 isolates were obtained. *Streptococcus* spp. had the highest prevalence rate (32%), while of 55 milk samples from Dairiboard factory, 71 isolates were obtained and *Bacillus* and Non-group D *Streptococcus* species were obtained in the highest number (20%). *Klebsiella* and *Citrobacter* were rarely encounted and accounted for 3.6% and 1.8% of isolates respectively. Tables 3 and 4 show the distribution of the isolates from different ice cream and milk products at different outlets. Results showed that products from A and B outlets had the highest number of isolates 26 and 14, respectively; while milk samples from outlets B and C recorded the highest bacteria isolate.

The median number of organisms per milliliter in the milk samples from Dorking Dairies milk product was 450 cfu/ml, while that from Dairiboard was 400 (cfu/ml). The interquartile range obtained from Dairiboard milk samples was 200 cfu/ml, 1000 cfu/ml; and that from Dorking Dairies milk was 100 cfu/ml and 3000 cfu/ml. Likewise the median number or organisms from vanilla and Strawberry ice cream samples were 50 cfu/ml and 100 cfu/ml respectively as summarized in Table 5. Results showed that there was no significant difference between growths of organisms in milk and ice cream obtained from the different sources ($p=0.42$ and $p=0.377$) respectively.

Dairy Regulation Act (1977) states that milk and ice cream for public consumption are required to pass the 30 min and 2 h methylene blue dye reduction tests respectively (Munri

TABLE 3. DISTRIBUTION OF ORGANISMS FOUND IN ICE CREAM WITHIN VARIOUS OUTLETS

Organisms	Vanilla ice cream outlets						Strawberry ice cream Outlets					
	Depot	A	B	C	D	E	Depot	A	B	C	D	E
Coagulase negative *Staphyloccocus*	-	1	-	-	-	-	-	-	-	-	-	-
Bacillus spp.	1	1	7	12	1	7	-	1	8	5	9	3
Micrococcus	1	-	1	2	2	2	-	-	2	-	-	-
Fusiform bacteria	-	-	-	4	-	3	-	-	-	1	-	-
Group D *Streptococcus* spp.	-	-	-	4	1	1	-	1	-	1	-	1
Non Group D *Streptococcus* spp.	-	-	1	4	-	1	-	-	1	1	-	-
Total	2	2	9	26	5	14	0	2	11	8	9	4

TABLE 4. DISTRIBUTION OF ORGANISMS FOUND IN MILK SAMPLES WITHIN THE OUTLETS

Organisms isolated	Depot	Milk samples from DD factory Outlets					Depot	Milk samples from DB factory Outlets				
		A	B	C	D	E		A	B	C	D	E
Coagulase negative *Staphylococcus* spp.	-	-	4	-	1	2	3	3	6	5	2	1
Bacillus spp.	-	2	3	3	1	1	1	4	6	1	2	4
Micrococcus spp.	-	3	2	-	5	5	-	3	2	1	2	-
Fusiform bacteria	-	-	-	-	-	-	-	3	2	1	2	1
Group D *Streptococcus* spp.	-	3	2	1	1	3	-	2	3	-	1	-
Non Group D *Streptococcus* spp.	-	3	2	-	1	-	1	2	1	2	-	-
Klebsiella spp.	-	-	-	-	-	-	-	-	1	1	-	-
Citrobacter spp.	-	-	-	-	-	-	-	-	-	2	-	-
Diptheroids spp.	-	-	-	-	-	-	-	-	-	2	-	-
Total	0	11	13	4	9	11	5	17	22	13	9	6

TABLE 5. NUMBER OF ORGANISMS OBTAINED FROM MILK AND ICE CREAM SAMPLES

Type of samples	Number sampled	Median cfu/ml	Interquatile range
DD milk	50	450	100, 3000
DB milk	55	400	200, 1000
Vanilla ice cream	46	50	0, 200
Strawberry ice cream	49	100	0, 200
Combined milk	105	400	100, 200
Combined ice cream	95	100	0, 200

et al. 1992). The results obtained from this study showed that all the milk and ice cream samples passed the methylene blue reduction test. This indicates that either the products were free from microbes or the microbial load was not significant to cause dye reduction. However, the plate count result of the milk and ice cream samples gave interquartile ranges of 100 cfu/ml, 2000 cfu/ml and 0 cfu/ml, 200 cfu/ml respectively. The reduction of the dye by some of the samples after a longer time suggests the presence of microbes in the dairy products. These organisms, given the appropriate environments could multiply and constitute health hazard to consumers. Samples analysed using the direct plate counts had pathogens such as *Klebsiella* spp, *Citrobacter* spp, Group D *Streptococcus*, which under the methylene blue reduction test had been considered acceptable. The fact that these samples passed the methylene blue reduction test makes the use of this test as the sole indicator of contaminated dairy products highly questionable.

The methylene blue dye is said to work best in samples whose predominant flora is mesophilic. Psychrotrophic bacteria, for example, are not active reducers of the dye where as *Streptococcus* spp. and the coliforms are. In the present study, samples containing *Streptococcus* spp. or coliforms had similar test result to those containing the non-pathogens such as *Staphylococcus* spp. or *Micrococcus* spp. It is possible that these pathogens were present in numbers that were too low to be detected by the dye. If this is the case, it is then best to use this test in conjunction with other tests to assess the microbial quality of milk and milk products.

Organisms isolated from the samples collected both from the factories and the outlets were similar. This implies that primary contamination of the products may have occurred at the manufacturing sites. Although the number of bacterial isolates was lower in the sample from the factories than those from the outlets. This is an indication that the multiplication of the organisms to detectable quantities, or further contamination of the products must have occurred in the outlets. This is in agreement with a previous study, which showed that contamination at the factory could occur through the air, in the holding tanks, pipes or the pasteurization room (Mahari and Gashe 1990). A comparison of the number of isolates obtained in the outlets revealed that some outlets had higher number of isolates than others. Given the inevitable prolonged storage of these products prior to sale, the storage temperature is likely to have effect on the microbial flora of the products. In addition, handing, transportation and general hygienic conditions in the outlets may have contributed to the contamination. In all the outlets examined it was not uncommon to find opened ice cream cups or leaking milk packets on display. The dairy regulation stipulates that all pasteurized milk and ice cream products should be free from all pathogens. In the present study, a number of pathogens and non-pathogens were isolated from both milk and ice cream samples. These included *Klebsiella* spp, *Citrobacter* spp., Group-D *Streptococcus* spp. *Micrococcus* spp. coagulase negative *Staphylococcus, Bacillus* spp. and *Diptheroids* spp. This result agrees with previous reports (Gill et al. 1994, Lindberg et al. 1998). Although, presence of these pathogens for example *Klebsiella* spp. (1%), *Citrobacter* spp. (14%) is low in the present study, their presence could contribute to spoilage, which could impose a serious health hazard as earlier reported (Lindberg et al. 1998). *Micrococcus* and *Bacillus* spp. are regarded as non-pathogens and their presence may only serve as an indicator of hygienic standard of dairy products, increase the plate counts and may possibly reduce the shelf-life and quality of the products they contaminate (Gill et al. 1994). These organisms were isolated 23(21.9%) of the milk and 10(10.5%) of the ice cream samples. It was evident from the plate count test that microbes were present in the dairy products examined. It was also established that the methylene blue reduction test may not be relied upon as the sole means of establishing contaminated dairy products. It is recommended therefore, to use the methylene blue reduction test in conjunction with other tests, such as the direct plate count method when assessing the bacteriological standard of dairy products. Previous study, (Sieva et al. 1993) has identified a number of possible sources that can increase the bacteria load of milk samples. These include inadequate refrigeration facilities, poor road conditions and high ambient temperature (Sieva et al. 1993). These were obvious poor conditions observed in the present study. It is therefore, suggested that all dairy products be transported in closed vans to minimize contamination. All ice cream cups found open or milk packets that are leaking should be rejected. It was not possible in this study to determine the role of adherence to expiration date on microbial contamination of products examined, because the products were all sold out before expiration dates.

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RESEARCH PAPER

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The Influence of Moisture Content and Temperature on Thermal Properties of Potato

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Studies were conducted with 'Kufri Chandramukhi' variety of potato to determine the thermal properties, i.e. specific heat, thermal conductivity and thermal diffusivity. Specific heat was determined at 6 levels of moisture content (0.33 to 4.88 kg/kg d.b.) and 5 levels of temperature (50 to 90^{0}C) by the method of mixtures. Thermal conductivity was determined at the same levels of moisture and eight levels of temperature (20 to 90^{0}C) by finite probe method and thermal diffusivity was calculated using standard relationship with specific heat, thermal conductivity and true density, at the levels of moisture and temperature same as that for specific heat. Effect of moisture and temperature on thermal properties was studied using multiple regression technique and the best fit was obtained by second order polynomial model. The effect of moisture content was found to be more pronounced than that of temperature for all the properties studied.

Keywords : Specific heat, Thermal conductivity, Thermal diffusivity, Method of mixtures, Finite probe method.

Knowledge of thermal properties of any food material is essential for modelling, optimization and designing of the process and equipment for all the operations based on thermal treatments like dehydration, blanching, cooking, steaming, frying, roasting and freezing (Szcezesniak 1983; Sweat 1986). Potato is the most widely consumed vegetable with highest annual production (17.65 million tonnes in 1997-98) among vegetables grown in India. (Anon 1999). It is preserved by freezing in cold-storage and wide ranging products are prepared commercially, based on thermal operations. However, substantial information is not available on the various thermal properties of potato, eg. specific heat, thermal conductivity and thermal diffusivity (Sweat 1986; Wang and Brennan 1992, 1993) and almost no systematic data could be found on common Indian varieties.

Several researchers have reported the moisture dependence of specific heat of a few varieties of potato tubers and established that specific heat increased significantly with moisture content and was observed to be in the range between 2 and 3.78 kJ/kg ^{0}C (Listov and Kalugina 1964; Yamada 1970; Lamberg and Hallstorm 1986). Specific heat of potato was reported to vary with temperature as well (Abdul-Rezzak 1983) as it increased from 2.7 to 4.0 kJ/kg ^{0}C, when the temperature was raised from 40 to 90^{0}C. Simultaneous effects of moisture content and temperature on specific heat of potato was also studied rather recently (Wang and Brennan 1993) and the following empirical model was suggested.

$$c_p = 1701.14 + 6.12T + 850.57M - 104.33M^2 \quad \text{..... (1)}$$

where, c_p = specific heat (J/kg ^{0}C), T = temperature (^{0}C) and M = the moisture content (kg moisture/kg solid, d.b.) of potato.

Thermal conductivity of potato was reported to be a function of temperature by various researchers (Yamada 1970; Abdul-Rezzak 1983; Lamberg and Hallstorm 1986). The thermal conductivities of different varieties of fresh potato were observed to vary between 0.485-0.556 W/m^{0}K for temperature range of 10-75^{0}C (Yamada 1970), 0.41-0.56 W/m^{0}K for temperature range of 40-90^{0}C (Abdul-Rezzak 1983) and between 0.648 -0.719 W/m^{0}K for temperature range 20-80^{0}C (Lamberg and Hallstorm 1986). Thus, a wide variation was observed in the values with the varietal difference. Thermal conductivity of potato was also observed to be a function of moisture content as it increased with increase in moisture content and was found to vary between 0.53 and 0.66 W/m^{0}K (Rao et al. 1975; Tschubik and Maslow 1973). Moisture dependence of thermal conductivity of potato was also reported by Wang and Brennan (1992), who further developed the following linear relationship between thermal conductivity and log of moisture content.

$$k = A + B \log M \quad \text{.....(2)}$$

where, k = the thermal conductivity of potato (W/m^{0}K) and A and B = the constants. However, they failed in their attempt to establish any relationship with temperature.

Thermal diffusivity, which basically is a function of specific heat, thermal conductivity and density, was determined earlier for potato, both experimentally and theoretically. The values were found to increase with temperature at various ranges and varied between 9.75×10^{-8} and 1.7×10^{-7} m^2/s depending upon the variety of potato (Mathews and Hall 1968; Rao et al. 1975; Rice et al. 1988).

Literature revealed that the thermal properties of potato would vary substantially with variation in moisture content and temperature and also varied with varietal difference (Tschubik and Maslow 1973). Therefore, a need was felt to generate data for one of the most popular Indian varieties of potato, viz., 'Kufri Chandramukhi'.

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Materials and Methods

Freshly harvested potato was procured, manually peeled and cut to uniform size (1 cm cubes). The average initial moisture content was determined to be 4.88 kg/kg (d.b.) by the oven method (Hall 1971), taking mean of 10 replications. The moisture content was varied to selected levels by thin layer drying at 50^{0}C with hot air velocity of 1 m/s. True densities of samples at various moisture levels were determined following liquid displacement method using hexane.

Specific heat : Specific heat values of potato at various moisture and temperature levels were determined by the method of mixtures (Mohsenin 1980). A 250 ml vacuum flask with 4 cm layer of thermocol insulation was used as calorimeter. A 26 gauge Cu-constantan thermocouple junction was inserted inside through the cap.

The heat capacity of the flask calorimeter (32.119 J/^{0}C) and the capsules (53.991 J/^{0}C) were predetermined. For determination of specific heat, both capsules were filled with identical amount of potato samples at the desired moisture content and were placed in the oven and equilibrated to the desired temperature level which was noted by multi-channel temperature indicator through the thermocouple placed inside the reference capsule. The test capsule was then quickly transferred inside the flask calorimeter containing measured volume of water and the lid was tightly replaced. The final temperature inside the calorimeter at equilibrium was noted.

Specific heat of the sample was determined by the following expression.

$$c_p = \frac{(H_f + W_w)\,(T_e - T_{cw}) - H_c\,(T_c - T_e)}{W_s\,(T_c - T_e)} \quad \ldots\ldots\ldots (3)$$

where H_f and H_c = heat capacities (J/^{0}C) of the flask and capsule respectively, T_{cw}, T_c and T_e = initial temperature of water, temperature of capsule and final temperature of mixture at equilibrium, respectively and W_s and W_w = the weights of potato sample and water (kg) in flask calorimeter, respectively.

Specific heat of potato was thus determined for 6 levels of moisture content (0.33, 0.54, 0.82, 1.22, 1.86 and 4.88 kg/kg d.b) and 5 levels of sample temperature (50, 60, 70, 80 and 90^{0}C), replicating each experimental condition thrice to obtain the mean specific heat and the simultaneous effects and relationship were statistically developed using multiple regression technique.

Thermal conductivity : Thermal conductivities of potato samples were determined following the finite probe method suggested by Sweat (1974). As recommended by Sweat and Haugh (1974) and Sweat (1986), the probe comprised of a brass tube 10 cm long and 1.0 mm diameter (length : dia ratio > 25). A 36 gauge Nichrome wire insulated with teflon and connected to a constant power source (1.5 V, 0.5 amp) was used as heat source. The temperature was sensed by a 26 gauge Cu-constantan thermocouple and recorded by a multi-channel data logger. The potato sample brought to required moisture level was packed inside a glass cylinder 2.5 cm diameter and 10 cm length and equilibrated in an oven to the desired temperature. The sample with glass cylinder was then quickly transferred inside a rectangular box fully insulated with glasswool and thermocol layers. The probe was inserted inside the core of the sample and the time of heating was pre-decided as 30 seconds for sample with 0.33 kg/kg d.b. of moisture and 60 seconds for the others, which was on the basis of maximum temperature rise of around 10^{0}C. Temperature was noted at an interval of 5 seconds and time vs. temperature curve was plotted. Thermal conductivity (k) was determined by the expression.

$$k = \frac{Q}{4\pi s} \quad \ldots\ldots (4)$$

where Q = the power supplied per meter length of probe and s = the slope of the time-temperature curve.

The setup was first calibrated using distilled water following the above method. Thermal conductivity determined this way is practically correct for the mean temperature within the probe heating range.

Mean thermal conductivity of potato samples were determined at 6 levels of moisture, same as that for specific heat and 8 levels of temperature (20, 30, 40, 50, 60, 70, 80 and 90^{0}C) with 3 replications for each experimental condition. These values were analyzed statistically using multiple regression technique.

Thermal diffusivity : Thermal diffusivity of potato samples were estimated at the same levels of moisture content and temperature as that for specific heat, using the following standard relationship.

$$\alpha = \frac{k}{\rho c_p} \quad \ldots\ldots\ldots (5)$$

where α = thermal diffusivity (m^2/s) and ρ = the true density of potato (kg/m^3) at the selected experimental condition.

Results and Discussion

Specific heat : The specific heat of potato was found to increase with moisture content as well as temperature as it varied form 2340 to 4030 J/kg^{0}C when the moisture content and temperature increased from 0.33 to 4.88 kg/kg d.b. and 50 to 90^{0}C, respectively. The simultaneous effect of moisture content and temperature on specific heat is represented graphically in Fig. 1.

The specific heat values thus determined were close to the earlier reported values (Yamada 1970; Abdul-Rezzak 1983). However, the values reported by Yamada (1970) were slightly lower (between 2070 and 3650 J/kg^{0}C) than that obtained in the present study for nearly the same range of variables (temperature, 40 to 90^{0}C, moisture content, 0.33 to 3.00 kg/kg d.b.). Some of the earlier studies (Abdul-Rezzak 1983; Halliday et al. 1994) indicated linear relationship of moisture and temperature with specific heat while second order polynomial relationship was also reported (Wang and Brennan 1992).

The observed data in the present study was found to

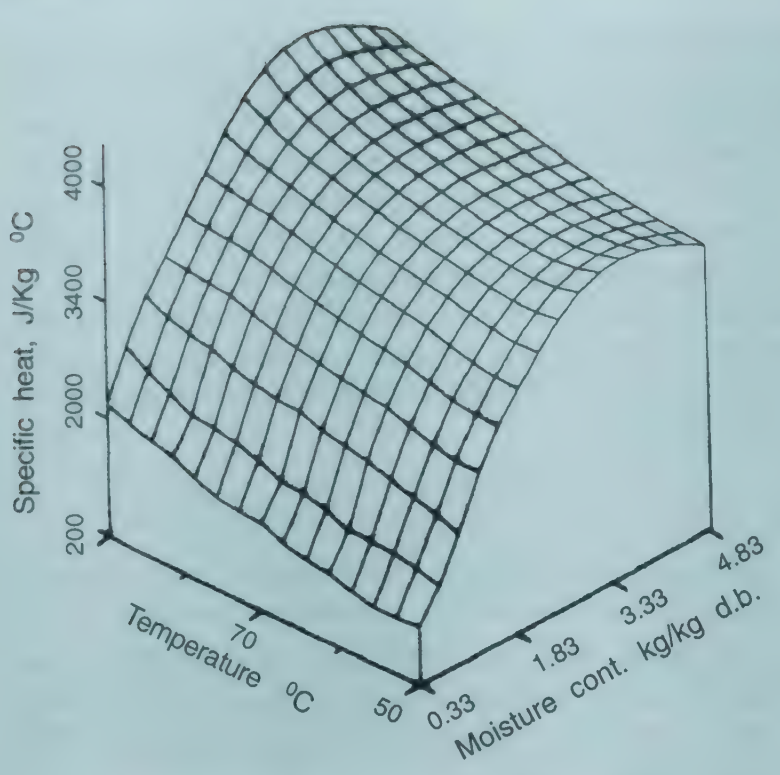

Fig. 1. Variation in specific heat of potato with moisture content and temperature

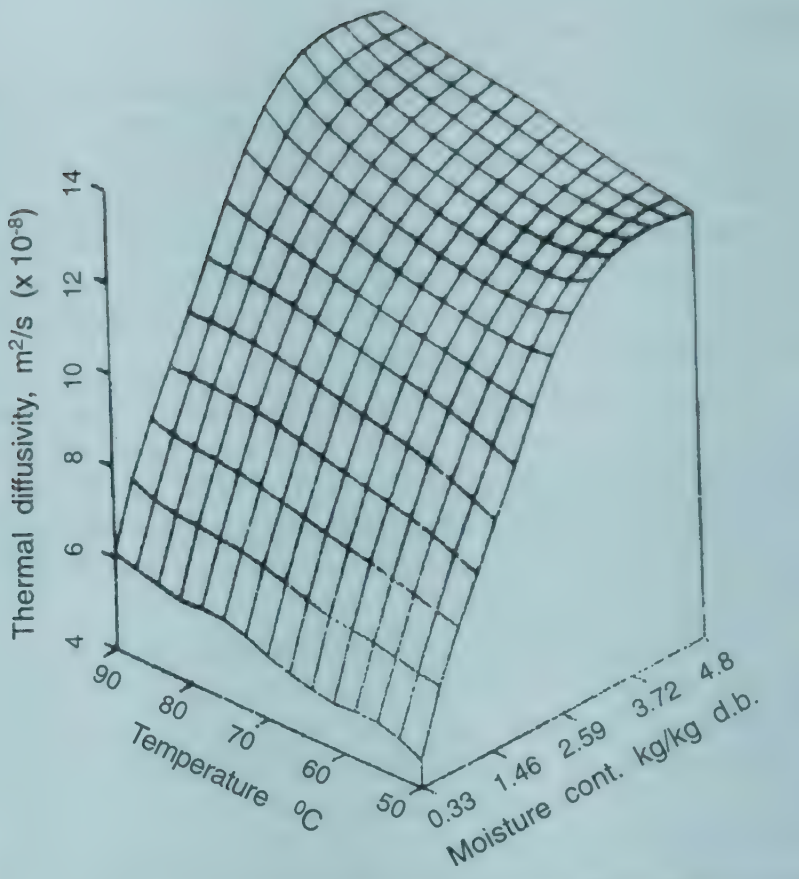

Fig. 3. Variation in thermal diffusivity of potato with moisture content and temperature

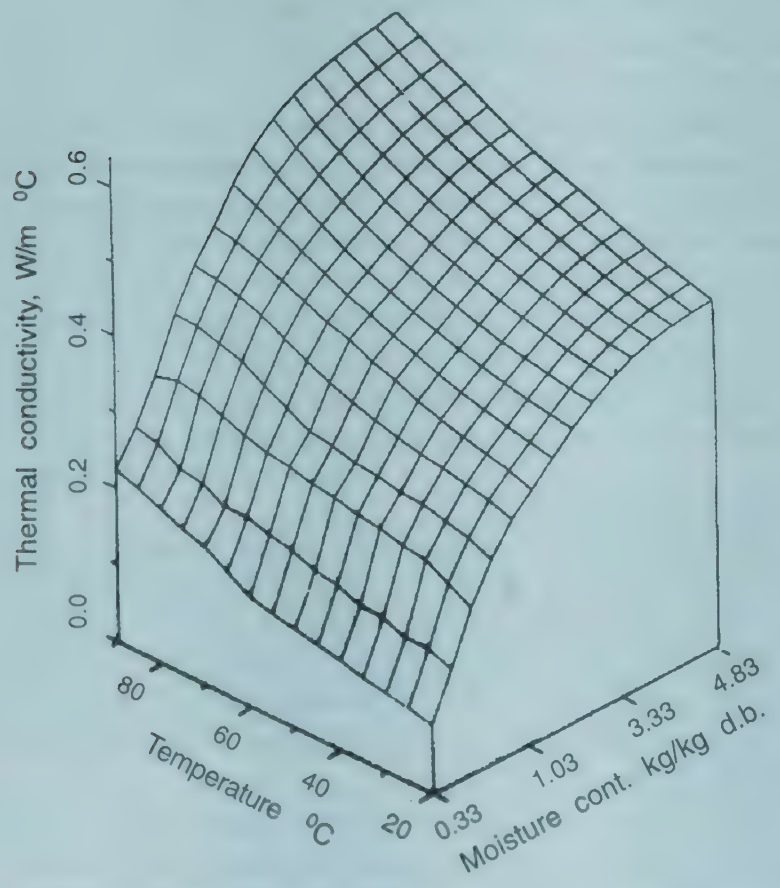

Fig. 2. Variation in thermal conductivity of potato with moisture content and temperature

fit well in the second order polynomial model with following relationship within the range of variables studied.

$$c_p = 2039.40 + 856.98\,M - 115.44\,M^2 - 0.069\,T^2 \quad (r = 0.987) \quad \ldots\ldots (6)$$

Specific heat showed a larger variation with moisture content as compared to temperature. The negative value of M^2 in eq. (6) suggested that the rate of increase of specific heat slowed down at higher range of moisture content. The analysis of variance presented in Table 1 showed non-significance of first order term of temperature, which indicated that temperature of the sample had a significant effect on specific heat only at a very high temperature range (T^2). The same effect could also be seen in Fig. 1. The ANOVA Table (Table 1) also showed that influence of M, M^2 and T^2 were all significant at 1% level with descending order of their sequence.

Thermal conductivity : Thermal conductivity of potato increased from 0.0889 to 0.6395 W/m^{0}C as the moisture content and temperature were simultaneously increased from 0.33 to 4.88 kg/kg (d.b.) and 20 to 90^{0}C, respectively. Larger variation was observed with moisture content as compared to temperature (Fig. 2) and the values determined exhibited close resemblance with that of the varieties reported earlier (Yamada 1970; Rao et al. 1975; Abdul Rezzak 1983). The more pronounced effect of moisture content on the thermal properties was possibly due to the reason that both specific heat and thermal conductivity of water were considerably higher than that of the air filled pores occurring at reduced moisture content (Jha and Prasad 1993).

The simultaneous effect of moisture and temperature on thermal conductivity of potato was found to be satisfactorily represented (r = 0.996) by the following second order polynomial model.

$$K = 0.0368 + 0.2008M - 0.0251M^2 + 2.3535 \times 10^{-4}\ MT + 1.4445 \times 10^{-5}\ T^2 \quad \ldots\ldots (7)$$

The estimated ANOVA (Table 2) showed non-significance of T indicating the influence of temperature only at a higher range (T^2). Both M and M^2 were found to be highly significant

TABLE 1. ANOVA OF THE MODEL FOR SPECIFIC HEAT OF POTATO

Source of variation	Sum of squares	DF	Mean sum of squares	F (cal.)
M	1634331.30	1	1634331.30	288.95**
T	2176.51	1	2176.51	0.33^{ns}
MxT	1023.25	1	1023.25	0.18^{ns}
M^2	899033.90	1	899033.90	185.95**
T^2	2420022.80	1	2420022.80	42.79**
Error	135746.40	24	5656.10	

ns=non-significant, ** significant at 1% level.

TABLE 2. ANOVA OF THE MODEL FOR THERMAL CONDUCTIVITY OF POTATO

Source of variation	Sum of squares	DF	Mean sum of squares	F (cal.)
M	0.0870	1	0.0870	316.2120**
T	0.2199×10^{-4}	1	0.2199×10^{-4}	0.1545^{ns}
MxT	0.0019	1	0.0019	7.0484*
M^2	0.0530	1	0.0530	254.1584**
T^2	0.0048	1	0.0048	17.4440**
Error	0.0116	42	0.0002	

ns=non-significant, *significant at 5% level, **significant at 1% level.

TABLE 3. ANOVA OF THE MODEL FOR THERMAL DIFFUSIVITY OF POTATO

Source of variation	Sum of squares	DF	Mean sum of squares	F (cal.)
M	58.5305	1	58.5305	1419.76**
T	8.2792	1	8.2792	200.83**
MxT	0.0107	1	0.0107	0.25^{ns}
M^2	22.6157	1	22.6157	548.59**
T^2	0.0023	1	0.0023	0.05^{ns}
Error	0.9894	24	0.0412	

ns=non-significant, **significant at 1% level.

(at 1% level) indicating the pronounced effect of moisture content at all levels. However, the rate of increase of thermal conductivity gradually decreased at higher levels of moisture content.

Thermal diffusivity : The true density values for estimation of thermal diffusivity were determined to be 1217, 1252, 1246, 1184, 1120 and 1070 kg/m^3 at the moisture contents of 0.33, 0.54, 0.82, 1.22, 1.86 and 4.88 kg/kg d.b. while the corresponding bulk densities were 388, 409, 434, 462, 508 and 632 kg/m^3 respectively. Thus, true density initially increased and later decreased with increase in moisture content and the maximum value was observed (1260 kg/m^3) at the moisture content of about 0.66 kg/kg d.b. (40% w.b.). This anomalous behaviour of true density may be accredited to the shrinkage of potato cubes during dehydration, which was substantial in the initial stages of drying and was rather insignificant in the later stage when the surface developed hard crust. Bulk density, on the other hand, increased continuously with increase in moisture content. As the experiments were conducted with samples packed inside and very little void space, true density was considered for determination of thermal diffusivity.

Thermal diffusivity thus calculated was found to increase with increase in moisture content as well as temperature and varied between 4.467×10^{-8} m^2/s (at 50^{0}C and 0.33 kg/kg d.b.) and 14.775×10^{-8} m^2/s (at 90^{0}C and 4.88 kg/kg d.b.) within the range of variables studied. The simultaneous effect of moisture content and temperature on thermal diffusivity of potato is graphically presented in Fig. 3 which also indicates the greater effect of moisture content on thermal diffusivity as compared to the temperature as well as the falling rate of increase of thermal diffusivity at higher moisture content. The values of thermal diffusivity obtained were reasonably close to the earlier reported values (Mathews et al. 1968; Rao et al. 1975).

The values showed considerably good fit (r=0.983) with second order polynomial model and the following relationship was derived.

$$\alpha(\times 10^{-8}) = 1.2844 + 4.5871M + 0.0372T - 0.517T8M^2 \quad ....(8)$$

The ANOVA for different terms in the model is given in Table 3. The significance of first order term of temperature and non-significance of second order term suggested distinctly the effect of temperature on thermal diffusivity and the nearly linear relationship, unlike that in the case of specific heat and thermal conductivity.

Thus, observations indicated that all the three thermal properties studied for potato increased with increase in moisture content and temperature and exhibited second order polynomial relationship. However, the influence of moisture content was greater in all the cases than that of the temperature.

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RESEARCH PAPER

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Development and Evaluation of Low Cost Coffee Pulper

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An attempt was made to develop a coffee pulper at a low cost with less water requirement so as to enable the small growers to follow wet processing method, which will give them more profit for their coffee beans. Three coffee pulpers, viz., (i) pulper with screw conveyor and concentric cylinders, (ii) pulper and (iii) pulper with cylinder and concave were designed, fabricated and evaluated for their pulping performance. The model-III pulper with cylinder concave mechanism was the best pulper and worked with a pulping efficiency of 98.0% and separation efficiency of 80.9%. The overall effectiveness of pulper-III was 0.793 and the performance was not affected even without water. The capacity of the pulper model-III was 80 kg/h.

Keywords : Coffee processing, Pulping, Pulper models, Pulping efficiency, Water requirement.

Coffee is a beverage made from the hot water extraction of solubles from the roasted and ground beans (seeds). Though the beverage has no nutritive value, it is consumed for its stimulating effects of caffein present in coffee ranging from 1.1 to 2.2%.

'Arabica' coffee and 'Robusta' coffee are the two important varieties of coffee cultivated in coffee growing countries. The tropical evergreen shrub grows at elevations between 700 and 2000 metres above the mean sea level at a temperature of 20^{0}C and an annual rainfall of about 1250 mm. The Indian production is two lakh tonnes per annum and export is 1.5 lakh tonnes every year (Anon 1993). Karnataka is the major coffee producer followed by Kerala, Tamil Nadu and Andhra Pradesh.

Harvested coffee fruits are processed by two methods: a) Wet method to prepare plantation or parchment coffee and b) Dry method to prepare cherry coffee.

The ratio of parchment to cherry coffee in 'Arabica' is 70 : 30 and 'Robusta' 6 : 94 (Menon 1989).

Preparation of parchment coffee by wet method requires pulping equipment and adequate supply of water. Pulping is the removal of epicarp (red skin) with the adhering mesocarp (pulp), which is done by squeezing the cherry between a moving rough surface and a stationary slotted surface to release the beans (Gumbe 1989). It has been reported that 40 cubic metres of water per tonne of clean coffee is required for receiving cherries, pulping and transporting the pulped beans and washing the beans (Rene Coste 1992). In India, there are more than one lakh small growers with below 4 hectares of land. It is not economically viable for these growers to buy commercially available pulpers and to provide large quantity of water for pulping (Terry Mabbet 1991). Therefore, an attempt was made to reduce the water consumption in pulping, and to develop a pulper at a low cost so that the small growers will process their coffee by wet method and get good returns for their coffee beans.

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The coffee fruit comprises the pulp, which houses the parchments, surrounded by a layer of mucilage (Plate 1). The fruits of 'Arabica' variety undergo green or wet processing in which the pulp is removed from the fruit by a pulping process. The mucilage is, then, removed by demucilaging and washing, and the parchments are dried in sunlight on the estate where they are grown.

The pulping should be done as soon as possible within 36 h of harvesting (Rene Coste 1992). The principle of pulping machines is to force the seeds to fly out through apertures, while the skin of the fruit is pulled beyond and thus separated (Haarer 1962).

In the ancient days, the cherries were pulped by hands or feet and the slimy parchment coffee were piled in heaps or into boxes. Mechanical pulping was developed during the early part of the 19th century and subsequently followed two basic designs, viz; the drum or cylinder type and disc type (Wrigley 1988).

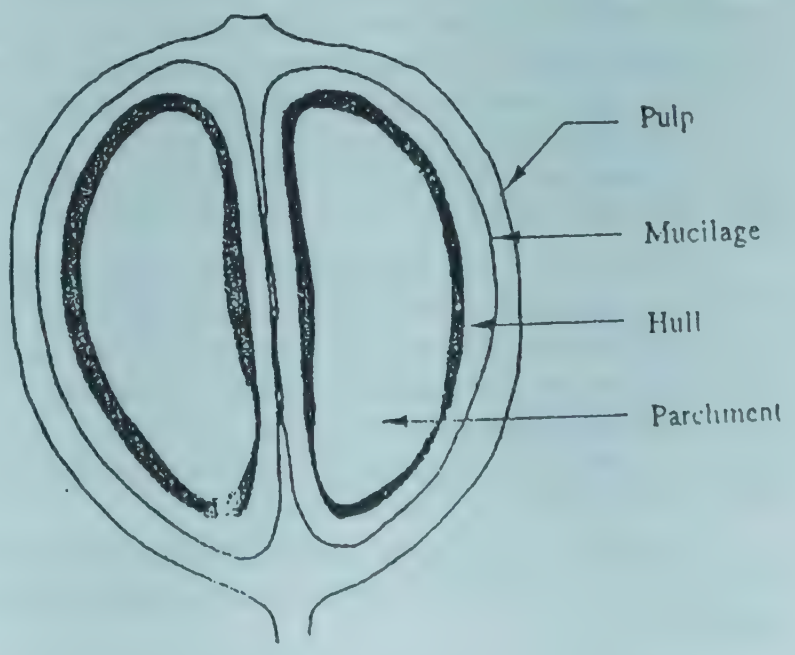

Plate 1. Cross section of coffee fruit

In 1845, John Gordon obtained a price for his iron pulper and subsequently took out a patent in 1858 for an improved breast that was the basis for future developments. In 1850, John Walker, a pioneer English inventor of coffee machinery, brought out his cylinder pulper for 'Arabica' coffee which became very popular in India and Sri Lanka (Wrigley 1988).

In 1860, Walker patented a disc type pulper. There is a further type of pulper, the Raeong or 'Aqua pulpa' which was developed in Indonesia and was designed to remove skin and mucilage in one operation. According to Wrigley (1988) the introduction of the Raeong pulper brought about a spectacular improvement in quality. The first local arabica coffee pulping machine was made by African artisans in 1920s (Nkonoki 1989)

Materials and Methods

To reduce the water consumption in pulping and cost of the pulpers, 3 models of pulpers were designed, fabricated and evaluated at the estate. The physical properties of 'Arabica' fruits like size, density, crushing strength, 1000 fruit mass and proportion of contents required for designing pulpers were taken from the data of Chandrasekar and Viswanathan (1999).

Coffee pulper - I

The major parts of the pulper were screw conveyor, inner cylinder, outer cylinder, feed hopper and a stand as shown in Fig. 1.

Screw conveyor : The flight of the screw conveyor was made of mild steel (MS) sheet of 10 SWG thickness for a length of 20.5 cm. The outer diameter of the screw conveyor was 19 cm and shaft was 2.54 cm. There were 5 screw flights at a pitch of 4 cm.

Inner cylinder : The inner cylinder was made of MS sheet of 20 SWG thickness with lips of 1 cm x 1 cm and 0.2 cm opening. The sheet was punched with a special die to form slot/lip of 1 cm x 1 cm area with 0.2 cm opening on one side. The outside diameter and length were 18 cm and 36 cm respectively with one side closed. This cylinder was mounted on the shaft over which the screw conveyor was fixed so that screw conveyor and inner cylinder will rotate simultaneously inside the outer cylinder.

Outer cylinder : The outer cylinder was made of same material used for making inner cylinder. It had two parts. The first part was having diameter of 25 cm and 20.5 cm length, inside which screw conveyor was rotated. The second part was a tapered cylinder with 25 and 20 cm diameter inside which inner cylinder was rotated. The two portions of the outer cylinder were joined by bolts and nuts.

Feed hopper : The feed hopper was made of MS sheet of 20 SWG thick. A feed regulator was provided to control the feed rate.

Stand : The stand was made of MS angle of dimensions 33 x 33 x 3 mm with a size of 75 cm x 33 cm x 60 cm height to support the pulper.

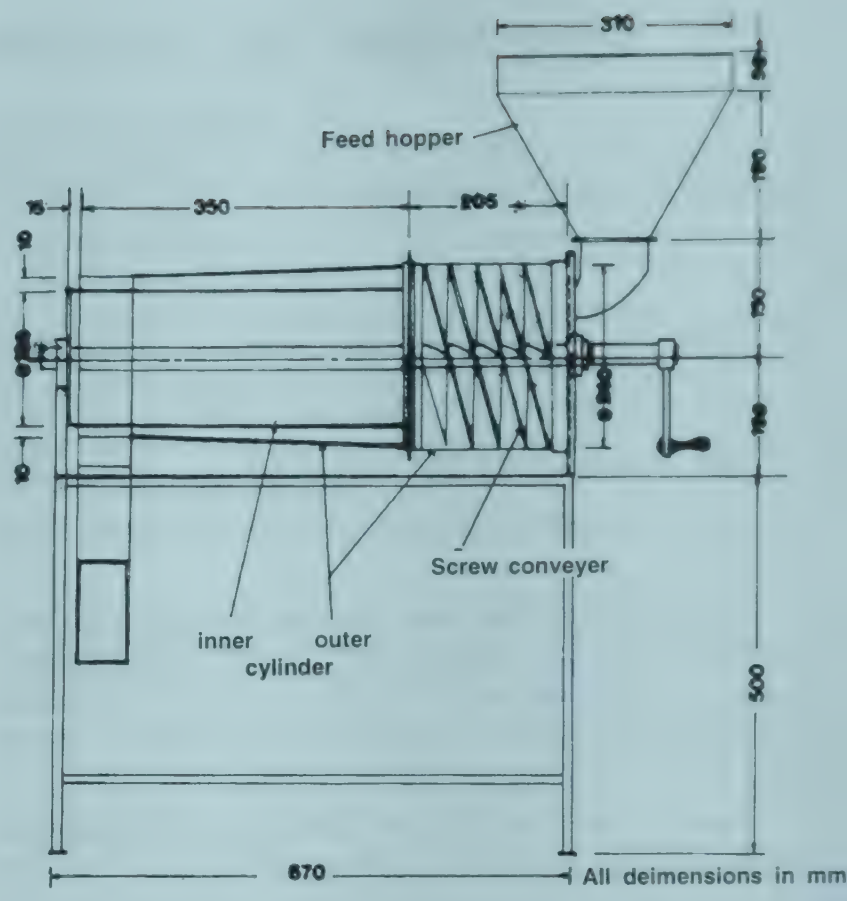

Fig 1. Coffee pulper model - I

Coffee pulper - II

This pulper consists of cylinder, concave, feed hopper and a stand as shown in Fig. 2.

Cylinder : The cylinder was made of MS sheet of 10 SWG thick. The outer diameter and length were 22 cm and 20 cm, respectively. This was fixed on a shaft of 2.54 cm diameter with a handle. A 20 SWG thick dimple sheet used in specific gravity separator was press fitted tightly over the cylinder.

Concave : Concave was made of number of 0.8 cm diameter MS rods fitted through the holes made on a MS flat of 2.54 x 0.8 cm. The gap between the two rods was 0.6 cm. The flat was bent to a shape of circular segment to a diameter of 23 cm. The concave was placed below the cylinder with a gap of 2 cm at inlet and 0.2 cm at outlet.

Feed hopper : Feed hopper was made of MS sheet of 18 SWG thick and the hopper was of tray type with 37 x 20 cm dimensions.

Stand : The stand was made of MS angle 28 x 38 x 4 mm the height was 85 cm with 35 x 35 cm square at the bottom.

Coffee pulper - III

The model consists of cylinder, concave, feed hopper, stand and handle as shown in Fig. 3.

Cylinder : The cylinder was 34 cm outside diameter and 29 cm long. The outer surface was made of rubber material. This was fitted over the shaft of 2.56 cm outside diameter with a handle. The outer surface was fitted with 6 numbers of MS

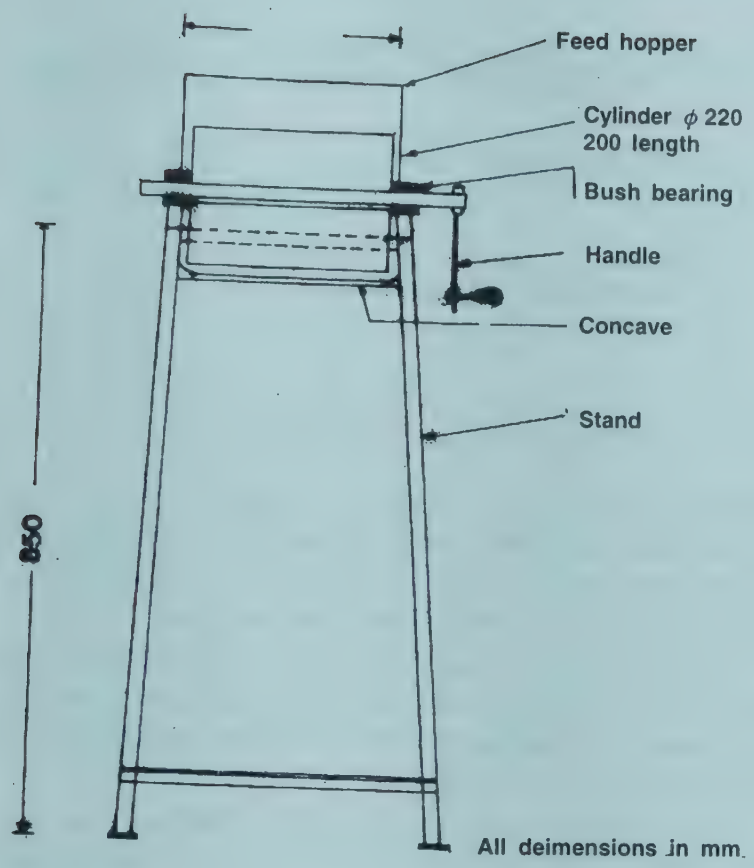

Fig 2. Coffee pulper model - II

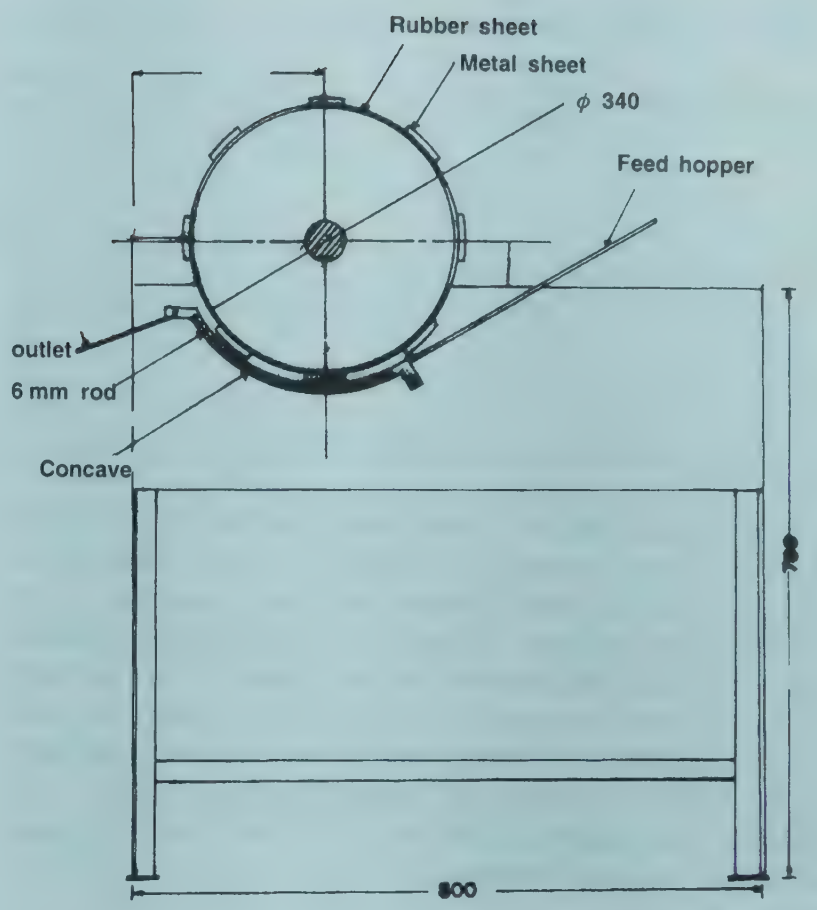

Fig 3. Coffee pulper model - III

sheet strips of 2 cm x 0.2 cm size throughout the length of the cylinder.

Concave : MS rods of 0.65 cm diameter were fitted on the holes made in the MS flat bend to a semicircular segment to a outside diameter of 34 cm. The gap between the rods was 6 mm. The concave was fitted below the cylinder with a gap of 2 cm at the inlet and 6 mm at the outlet end. Provision to change the gap at the outlet and the inlet end was provided.

Feed hopper : Feed hopper was made of MS sheet of 20 SWG thick and the hopper was of tray type with 31 x 31 cm dimensions.

Stand : Stand was made of MS angle of 55 x 55 x 6 mm. The stand has the length of 78 cm, breadth of 43 cm and height of 75 cm.

Evaluation of pulper

The three models of coffee pulpers were evaluated at the Horticultural Research Station, Tamil Nadu Agricultural University, Yercaud and Coffee Demonstration Farm, Coffee Board, Yercaud with 'Arabica' fruits.

Harvested fruits were fed into the pulpers at uniform rate and rotated manually at a speed of 50 to 60 rpm. Measured amount of water was added for efficient pulping and conveyance of skin and beans. Then bean, skin, broken beans and unpulped fruits were separated manually to evaluate the effectiveness of pulping.

The performance of the pulper was evaluated using the following equations :

1. Pulping efficiency $E_p = P/W \times 100$

   Where P - Quantity of fruits pulped in kg/min
   W - Quantity of fruits fed in kg/min
   E_p - Pulping efficiency in percentage.

2. Separation efficiency $E_s = \dfrac{ad - bc}{(a + b)(d + c)} \times 100$

   Where a - Quantity of beans in bean outlet, kg/min
   b - Quantity of skin in bean outlet, kg/min
   c - Quantity of beans in the skin outlet, kg/min
   d - Quantity of skin in skin outlet, kg/min
   E_s - Separation efficiency in percentage.

3. The overall effectiveness of the pulper = $E_p \times E_s \times 10^{-4}$

4. The capacity of the pulper $C = \dfrac{P}{T}$

   Where C - Capacity in kg/min
   P - Quantity of fruits pulped in kg
   T - Time taken in min.

Results and Discussion

Coffee pulper - I

The coffee fruits along with water fed from the hopper was conveyed by the screw conveyor and was forced to enter into the annular space between the inner and outer cylinders. As the annular space between the two cylinders were decreasing

TABLE 1. RESULTS OF COFFEE PULPER - III AT 6 MM CONCAVE CLEARANCE

Sl.no	Outlet clearance mm	Water consumed lit/min	Capacity kg/hr	Broken %	Pulping efficiency	Separation efficiency	Overall effectiveness
1	6	0	80	5	100	69	0.69
2	6	1.5	80	5	100	79	0.79
3	8	0	78	2	98	80.9	0.793
4	8	1.5	80	2	90	88	0.793
5	10	0	80	2	90	88	0.792
6	10	1.5	78.8	2	90	88	0.792

from 3.5 cm at inlet to 1 cm at outlet the fruits were crushed and beans were squeezed out of skin. The beans being bigger than the slots of inner cylinder, they were conveyed in the annular space between cylinders and collected at the outer end. The skin was expected to get crushed to fine particles so that it passes through the slots of inner cylinder and falls down separately. But, at the end, the skin took the same path of the beans and came out through the bean outlet. The machine has pulped all the fruits but the skin and bean separation was not effective.

Coffee pulper - II

The coffee fruits entered between the concave and cylinder as the handle was rotated. As the gap between concave and cylinder was reducing towards outside, the entered fruits were squeezed and bean came out of skin. The bean fell down through the gap between the roads of concave, whereas the skin which was attached to the lip surface was carried along by rotation and fell down away from concave.

This pulper pulped all the fruits effectively and separated skin and beans even without addition of water. But, the beans were damaged by the dimples on the periphery of rotor which was very sharp. Hence the beans obtained were either broken or mucilage and silver skin removed.

Coffee pulper - III

The material fed from the hopper entered through the gap between the cylinder and concave. As the gap was becoming narrow towards the outlet, the bean was squeezed out of the skin and fell through the gap between the rods. The skin was carried by the metal strips along the cylinder and collected at the outlet. As the surface of the cylinder was coated with rubber the breakage of beans was avoided.

During the evaluation trials with model-III, both pulping and separation of beans and skin were found to be effective. Hence, further trials were conducted by varying the operating parameters like clearance and flow rate of water.

The initial trails were conducted with concaves made of mild steel round rods with clearance between the rods of 8 mm, 7 mm and 6 mm. The clearance between the concave and cylinder (outlet clearance) were also varied. Among 8, 7 and 6 mm clearance between rods, trails showed that with 8 and 7 mm concave clearance most of the fruits came out of the clearance between rods unpulped. Hence, only for 6 mm concave clearance, trails were conducted varying the outlet clearance (clearance between cylinder and concave) at 6, 8 and 10 mm at skin outlet. The data are given in Table 1.

At 6 mm outlet clearance, the pulping efficiency was 100% with 5% broken beans and 69% separation efficiency without water. The separation efficiency increased to 79% with water at the rate of 1.5 lit/min.

At 8 mm outlet clearance, the pulping efficiency was 98% and separation efficiency was 80.9%. The capacity and separation efficiency did not vary significantly by addition of water.

At 10 mm outlet clearance, the pulping efficiency decreased to 90% and separation efficiency increased to 88%. The overall effectiveness was unaffected by addition of water.

Conclusion

Overall, the pulper model - I which was fabricated with lip type perforation and screw conveyor pulped the coffee fruits effectively but the separation of skin and bean was not effective.

The model - II, with cylinder and concave pulped the beans effectively and also separated the beans and skins. But, due to the sharpness of dimple sheet used in cylinder the beans were damaged. Hence, in model - III, the rubber coating was provided over the cylinder to avoid damage to the beans. This pulper - III worked well with capacity of 80 kg/hr. The pulping efficiency was 98% and separation efficiency of 80.9% even without addition of water with an out let clearance of 8 mm. The pulper performance was not affected by addition of water. Hence, pulper- III was found as the best pulper among three models with maximum effectiveness and minimum breakage of beans.

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RESEARCH PAPER

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Biochemical Changes During Iced Storage of Indian Milk Fish (*Chanos chanos*)

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Milk fish collected live from brackish water culture ponds were used for the ice storage studies. Biochemical parameters like changes in sulphydryl (SH) group, salt soluble nitrogen (SSN), free amino acids (FAA), alpha amino nitrogen (ANN) and electrophoretic studies were carried out. During iced storage, levels of alpha amino nitrogen, non protein nitrogen, total nitrogen, salt soluble nitrogen and SH groups decreased. Taurine, glutamic acid, glycine and histidine which contributed to 87% of the total FAA, decreased to a great extent, which also lead to decreased acceptability. Salt soluble nitrogen as percentage of total nitrogen increased. High correlation was observed between alpha amino nitrogen and total free amino acids and between salt soluble nitrogen and SH group.

Keywords : Alpha amino nitrogen, Free amino acids, -SH groups, Solubility, Non-protein nitrogen, *Chanos chanos*, Milk fish.

Twenty nine percent of the world fish catch is chilled and consumed as fresh and icing is one of the best preprocessing practices. The preservation of fish by icing is important for extending the shelf life of fish. Post-harvest losses are known to occur largely in tropical areas. Fish is more susceptible to spoilage compared to land animals. Hence it should be properly preserved and processed so that post harvest losses are reduced significantly.

During iced storage many changes occur in the fish muscle component and there can be changes in proteins, lipids, vitamins, minerals, non-protein nitrogen fraction, sulphydril group etc. and the quality of the product is impaired for further use due to protein denaturation (Reddy and Srikar 1991a). These changes are mainly temperature dependent and the changes in protein are important both from the point of view of nutrition and for proper utilization of fish. Free amino acids are known to be responsible for flavour and taste of fresh fish muscle (Sakaguchi et al. 1984). Loss of free amino acids during iced storage was reported earlier (Bramsnaes 1961; Basu and Khasim 1985).

Changes in sulphydryl (SH) and SS bonds play a major role in structure and function of native protein and also play critical role in the functional properties of proteinatious foods (Wolf 1970; Itoh et al. 1979). Buttkus (1971) reported a decrease in total SH groups of mysoin prepared from trout and rabbit during storage at 0^0C, with concomitant decline in adinosine triphosphatase activity and formation of aggregates. Joseph et al 1980 studied the changes in protein and non protein nitrogen fractions during iced storage of *Chanos chanos*. But the changes in SH groups and free amino acids, which indicate the loss in protein solubility and flavour have not been dealt with. Therefore, an attempt was made to study all the above biochemical changes during storage of *Chanos chanos* in ice and to understand the correlation between them.

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Materials and Methods

The study was carried out using milk fish (*Chanos chanos*) collected live from the brackish water culture ponds near Kochi, which were iced immediately. These were brought to the laboratory and kept for iced storage studies. The length of the fish ranged from 25-30 cm and the weight from 200-235 g. Fish to ice ratio was 1 : 2 and the experiment was carried out in an insulated rectangular box kept at room temperature. Melted ice was replenished everyday. Samples were drawn once in three days for analysis. The whole muscle from these fish were pooled and minced carefully to avoid heat generation and taken for analysis.

Moisture and total nitrogen were determined according to AOAC (1975). Extracts of the samples were prepared by centrifuging 3 g of accurately weighed sample homogenized in a polytron homogenizer with 60 ml of precooled Dyer's buffer (5% NaCl buffered with 0.02 M $NaHCO_3$) (Dyer et al. 1950). Centrifugation was done at 10,000 rpm for 20 min at 0^0C. The supematant was used for determination of salt soluble protein (Gornel et al. 1949) and salt soluble nitrogen (SSN) (Dyer et al. 1950). Sulphydryl group was estimated as described by Sedlak and Lindsay (1968). Trichloro acetic acid (TCA) extract was prepared by grinding 10 g of accurately weighed sample with 10% TCA and the content was quantitatively filtered through Whatman filter paper No. 1 and the volume was made upto 100 ml with 10% TCA. The TCA extract was used to measure alpha amino nitrogen (AAN) (EBC 1975), non protein nitrogen (NPN) (AOAC 1975) and total free amino acids (FAA) which was estimated after adjusting the pH of the TCA extracts to around 2.5 and separating the individual amino acids by HPLC (Ishida et al. 1981). Total amino acids were estimated by acid hydrolysis with 6 N HCl of about 100 mg of the fish muscle for 24 h at 110^0C in vacuum sealed tubes. The sample was then filtered and flash evaporated to remove the acid, then the pH was adjusted to around 2.5 and separation was done by HPLC (Ishida et al. 1981). Tryptophan was estimated by colorimetery after alkali hydrolysis as per the method of Sastry

TABLE 1. CHANGES IN TN, SSN, NPN, AAN, TOTAL FAA (EXPRESSED AS MG/G DM) AND SH GROUP DURING ICED STORAGE OF *CHANOS CHANOS*

Storage period, days	Moisture %	AAN	NPN	Total FAA	TN	SSN	SH groups µ mol/g DM
0	77.63	6.2 ± 0.50	21.5 ± 2.20	48.00	154.2 ± 4.40	136.7 ± 2.7	85.1 ± 6.80
4	78.94	5.7 ± 0.60	19.8 ± 2.00	41.10	152.7 ± 3.00	135.0 ± 7.3	77.6 ± 1.16
7	78.53	5.5 ± 0.60	17.9 ± 0.18	37.50	150.2 ± 5.80	133.7 ± 6.7	83.8 ± 1.16
11	80.58	4.3 ± 0.24	13.4 ± 0.18	33.30	148.6 ± 3.20	133.2 ± 5.6	80.6 ± 8.80
14	81.40	3.4 ± 0.46	10.4 ± 1.10	24.00	147.0 ± 3.20	132.3 ± 3.9	80.2 ± 1.60
18	81.40	3.6 ± 0.02	11.8 ± 0.90	24.70	141.8 ± 2.80	129.1 ± 9.0	78.6 ± 1.57
21	79.40	2.6 ± 0.51	8.1 ± 0.80	19.90	126.6 ± 2.60	117.0 ± 5.9	70.8 ± 1.90

± SD = Standard deviation. n = 3

TN = Total nitrogen
AAN = Alpha amino nitrogen
AA = Amino acid
NPN = Non protein nitrogen
SSN = Salt soluble nitrogen
FAA = Free amino acid
DM = Dry matter

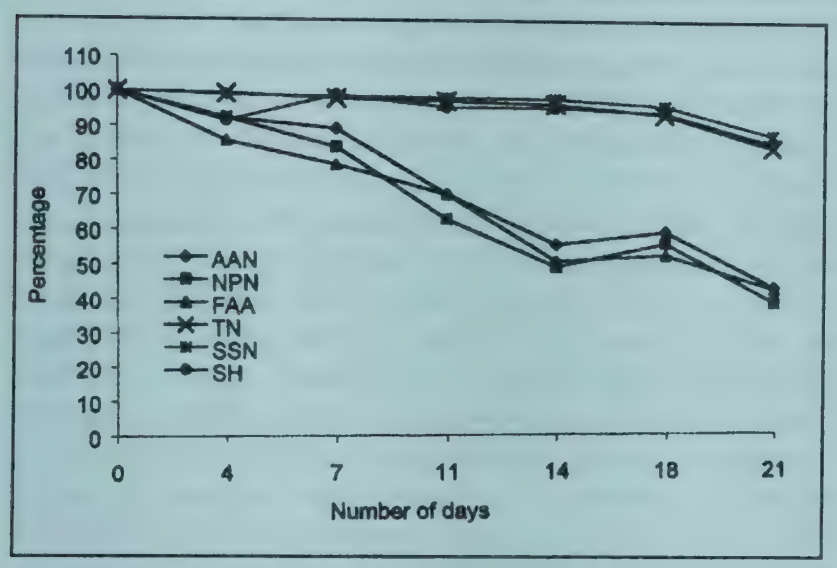

Fig. 1. Retention % of TN, NPN, AAN, FAA & SH group during iced storage of *Chanos chanos* (based on the initial value). TN - Total nitrogen, NPN - Non protein nitrogen, AAN - Alpha - Amino nitrogen, FAA - Free amino acids, SH - Sulfhydryl

and Tummuru 1985. Correlation analysis was done using a standard statistical software viz. Statistical Package for Social Sciences. All the determinations were carried out in triplicates except amino acids and moisture, which were only in duplicates.

Results and Discussion

Chanos chanos had 74 to 77.6% of moisture, 19 to 20% of protein, 1.2 to 1.6% of ash and 1.6 to 2.0% of fat. Changes in the moisture content, AAN, NPN, total FAA, TN, SSN, SH group of the fish during ice storage are shown in Table 1. A gradual increase in moisture from 77.6 to 81.4% was observed upto 14 days of iced storage then remained more or less same upto 18 days and afterwards showed a slight decrease in moisture content (79.4%). The initial increase in moisture may be due to the absorption of ice melt water by the muscle. Basu and Khasim (1985) found a decrease in moisture content for 3 days, followed by a gradual increase upto 19 days and on further storage the moisture content decreased. The decrease in moisture content at the later stages of ice storage was attributed to the softening of the muscle during prolonged storage and exudation of water from the tissue due to the pressure of ice (Partman 1965; Basu and Khasim 1985). The loss of water holding capacity due to changes in protein in the later stages of iced storage may also be a reason for the decrease in moisture content. The values for NPN showed a decreasing trend, except for a slight increase on the 18th day. At the end of 21 days, the NPN value reduced to 37.5% of the initial value. Due to autolysis and microbial growth during chill storage an increase in NPN is expected. These water-soluble compounds are leached out along with water and thus their decrease during storage in ice is expected. The decrease may also be due to the utilization of the FAA by the bacterial flora. The decrease in total nitrogen content observed may be mostly due to the decrease in NPN.

Fig. 1 gives the percentage reduction in AAN, TN, NPN, total FAA and SH groups. A gradual decrease in AAN was observed during storage in ice, which was reduced to 55.4% of the initial value by 21 days (Fig 1). Correspondingly there was a loss of 58.6% in the total free amino acids. In fish packed in polythene bags and stored in ice, an initial decrease in AAN upto 9 days, followed by an increase was observed (Nair et al. 1971). The decrease in AAN in packed fish can be attributed to bacterial growth and to deamination reaction (Nair et al. 1971). During ice storage, decrease in AAN, due to leaching of water soluble compounds seems to be higher than their increase by proteolysis. The decrease in AAN significantly correlated with the total free amino acid content and NPN.

The changes in the major free amino acids are given in Table 2. Taurine, glutamic acid, glycine, alanine, lysine and histidine together contributed 94.7% of the total free amino acids while all other amino acids together contributed to the remaining 5.3% in the 0 day sample. But the percentage of the major amino acids decreased to 73.6% by 21 days iced storage. Among the major amino acids only glutamic acid

TABLE 2. CHANGES IN MAJOR FREE AMINO ACIDS DURING ICED STORAGE OF *CHANOS CHANOS*

Amino acid, mg/g DM	0th day	4th day	7th day	11th day	14th day	18th day	21st day
Taurine	8.48	6.02	5.57	3.48	2.52	1.72	1.11
Glutamic acid	1.30	1.61	1.59	1.30	0.95	1.02	0.90
Glycine	7.00	4.58	3.48	1.81	1.41	1.12	0.54
Histidine	25.07	21.86	20.52	16.93	11.95	11.03	8.98
Alanine	1.44	0.84	1.03	0.79	0.62	1.36	0.52
Lysine	2.16	2.28	2.34	2.98	2.46	3.21	2.60

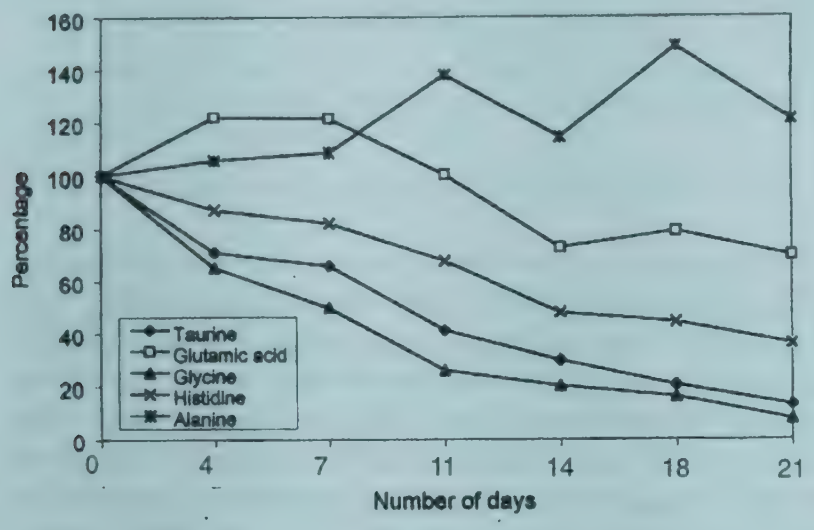

Fig. 2. Retention % of major free amino acids during the iced storage of *Chanos chanos* (based on the initial value)

showed slight increase upto 7 days. Lysine, in the free amino acid pool, increased upto 18 days and then decreased but 21 days stored samples showed higher value than the initial. Taurine, glycine, alanine and histidine decreased gradually retaining only 13%, 7.7%, 36% and 35.8% respectively of their initial values at the end of storage (Fig 2). Other amino acids found to decrease during storage were serine and methionine. Levels of aspartic acid and proline steadily increased. The rest of the amino acids showed insignificant changes during storage.

Cod muscle peptidases are known to split small peptides and release amino acids like glutamic acid, aspartic acid, valine, phenylalanine, leucine, and lysine (Bramstedt 1961). Murata and Sakaguchi (1988) found that glutamic acid decreased in the intermediate period (20 days), during iced storage for 40 days. In their studies alanine increased markedly in the early period and arginine decreased after 22 days while the other amino acids showed no measurable changes. The drop in the level of most of the FAA during iced storage can be attributed to bacterial spoilage. When juvenile mackerel muscle was packed in plastic vials and stored in ice, Sakaguchi et al (1984) observed that histidine and taurine content of white muscle decreased. Aspartic acid and tryptophan levels decreased initially but subsequently their levels increased. On the other hand, arginine and glutamic acid levels increased during the first half of iced storage but subsequently their levels declined rapidly. Compared to the present study there was no leaching of these amino acids in the ice melt water, since the fish muscle was not directly in contact with ice.

Umar and Qadri (1988) found that FAA decreased and then increased in some species viz. white pomfret, catfish and flat fish and decreased gradually in other species viz. snapper and Indian shad. The present results for *C. chanos* showed that isoleucine, leucine and valine decreased gradually while no individual FAA was found to accumulate during iced storage.

Salt soluble nitrogen decreased from 136.7 to 117 mg/g DM (Table. 1) within 21 days of storage in ice. But, the salt soluble nitrogen, expressed as percentage of total nitrogen did not show significant variations initially but later showed a gradual increase. In *C. chanos* stored for 18 days in ice, Joseph et al (1980) found SSN as % of TN to decrease gradually with a loss of 9.4% at the end of storage. The present study showed a decrease of 19.7 mg SSN/g dry matter. But, with a loss of 27.8 mg TN/g dry matter, the SSN expressed as % TN showed an increase. Castell et al (1973) found extractable protein nitrogen as % of muscle protein to increase in pollock, cod and cusk during iced storage. When proteolytic degradation occurs, the compact, high molecular weight myofibrillar proteins are hydrolysed to low molecular weight proteins or peptides. These degraded proteins might have relatively higher solubility than their native myofibrillar proteins. Low temperature employed during preprocessing and processing procedures reduce but do not completely inhibit proteolysis (Lin and Park 1996). Studies on changes in the solubility of protein of different species of fish during storage in ice have been reported (Reddy and Srikar 1991b; Sarma et al. 1999; Srikar 1979; Reddy and Srikar 1991a). In these studies the decrease in solubility of proteins was attributed to leaching of water-soluble proteins, aggregation of proteins and insolubilisation caused by the oxidised and hydrolysed products of lipids through hydrophobic association.

A gradual reduction of SH groups from 85 μmoles/g dry matter to 70.6 μmole/g dry matter was observed (Table 1). Not

TABLE 3. CORRELATION COFFICIENT BETWEEN AAN, NPN, FAA, TN, SSN, SH GROUP DURING ICED STORAGE OF *CHANOS CHANOS*

Parameters	AAN	NPN	FAA	TN	SSN	SH group
Alpha amino nitrogen	–	0.991***	0.988***	0.845*	0.805*	0.735
Non protein nitrogen	–	–	0.980***	0.845*	0.799*	0.689
Free amino acids	–	–	–	0.825*	0.782*	0.700
Total nitrogen	–	–	–	–	0.995***	0.861**
Salt soluble nitrogen	–	–	–	–		0.879**

***Significant at $P < 0.001$, **$P < 0.01$, *$P < 0.05$, others have no significant correlation.

TABLE 4. CHANGES IN THE ESSENTIAL AMINO ACIDS CONTENT DURING ICED STORAGE OF *CHANOS CHANOS*

Amino acid, mg/g DM	Fresh	Stored in ice for 21 days
Threonine	44.57	40.96
Cysteine	16.5	14.76
Methionine	20.7	20.8
Valine	48.4	46.0
Isoleucine	40.18	39.2
Leucine	75.04	72.0
Tyrosine + Phenylalanine	69.05	62.7
Histidine	60.69	41.2
Lysine	64.13	60.8
Tryptophan	11.58	10.60

* Cysteine and Tyrosine can spare the essential amino acids methionine and phenylalanine, so these are included in the Table

* Histidine is essential for children.

much work has been reported earlier on the fate of SH groups of fish muscle during iced storage. The loss in SH groups in the study was not due to leaching of the amino acids since cysteine was not detected in the free amino acid pool throughout the storage period.

During a series of studies conducted by Sompongse et al (1996 a, b, c, d) on the stability of carp actomyosin during ice storage, it was found that the decrease in the SH content was due to the oxidation of SH groups. Benjakul et al (1997) observed that the total SH content of Pacific whiting muscle protein increased slightly after 2 days of storage in ice, followed by a gradual and continued decrease upto 8 days. In this study, only a gradual decrease was noticed, which may be due to the fact that the first sampling was done only on the fourth day. The reduction in SH group was around 17%.

The correlations between various chemical parameters are given in Table 3. There was highly significant correlation between AAN, NPN and FAA. Highly significant correlation was also found between TN and SSN. But there was no significant correlation of SH group with AAN, FAA and NPN.

Table 4 gives the changes in the essential amino acids content by 21 days storage in ice of *C. chanos*. Most of the essential amino acids content decreased slightly but the changes were not statistically significant. This loss may also be due to leaching.

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RESEARCH NOTE

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Processing of Ou-tenga (*Dillenia indica*) Fruit for Preparation of Squash and Its Quality Changes During Storage

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Three methods were tested for preparation of squash from ripe ou-tenga (*Dillenia indica*) fruit at its optimum maturity stage. Initial pH range of 1.37 to 2.61 of the squashes prepared was increased to 2.24 to 2.88 after 60 days of storage. Total sugar percentage range at 0 day (26.54 to 48.48%) was also increased to 31.15 to 55.05% at 60 days of storage. pH (1.37), TSS (48.0%), acidity (0.65%) and total sugars (48.48%) were found to be an appropriate combination contributing to organoleptic qualities of the squash prepared by the Method A. Organoleptically also Method A was found superior with scored values of colour (9.6±0.4) taste (9.4±0.6) and astringency (4.2±0.8). However, flavour score in Method A (9.4±0.6) was found lower than method B (9.8±0.2). Significant effects of storage were observed on pH, TSS, titrable acidity, reducing sugars, total sugars and non-reducing sugar. Significant effect of storage was also found in organoleptic parameters of colour, flavour, taste and astringency.

Keywords : Processing, Squash, Storage, Bio-chemical, Organoleptic parameters, Preparation methods, Indigenous, *Dillenia indica*

Ou-tenga (*Dillenia indica*) is one of the most important minor fruits of Assam. It is a handsome evergreen tree, 30-80 feet in height which bears large and hard fruit of 3-5 inches in diameter, consisting of 5 closely fitted imbricate sepals enclosing numerous seeds embedded in a glutinous pulp (Sastri 1952). The ripe fruits are gathered while the sepals thickened, which are sour in taste and widely used in flavouring of curries and in the preparation of jam and jelly. The acid juice sweetened with sugar is used as cooling drink. It is rich in protein, carbohydrate, fat and fibre with fairly high amount of calcium, phosphorus and energy value of 59 Kcal per 100 g (Gopalan et al. 1971).

Ou-tenga fruit could be processed to commercial products some of which have been reported like clear beverage (Vaidehi and Annapurna 1985) and Ready to Serve (RTS) beverage (Saikia and Dutta 1995). From a study on physico-chemical and organoleptic changes of ou-tenga fruit with growth and development, it was found that for the preparation of commercial products with optimum flavour, colour and nutrient content the best harvesting index was after 160 days of fruit set (Saikia and Dutta 1991). For higher juice recovery, blanching of cut sepals for 8 min. in boiling water was found to be optimum (Saikia and Dutta 1993). As ou-tenga fruit grown in the whole North East India, is a minor fruit with excellent flavour and taste, it has to be popularized further with the preparation, standardization and commercialization of its products like squash. Research works on the preparation of squash, its bio-chemical, organoleptic quality and storage have not been done so far, therefore, this study was undertaken in an ICAR ad-hoc scheme.

Ou-tenga (*Dillenia indica*) fruits were collected after 160 days of fruit set at optimum maturity levels as described by Saikia and Dutta (1995). Optimum maturity was obtained by the fruit when it developed dusky yellow colour coinciding with highest scores value for flavour, taste, astringency and bio-chemical composition of important parameters. Fruits were cleaned in water, air dried manually in the laboratory and squashes were prepared by three methods mentioned below.

Method A : Small washed segments (2 kg) of ou-tenga fruits were cooked with water (1 litre) under pressure of 15 lb for 15 min. cooled by removing the lid of the cooker. Juice from the segments was extracted by using a Mather and Platt screw type juice extractor. Ou-tenga juice (extract) so prepared was mixed with remaining water of pressure cooker, strained through muslin cloth. Measured quantity of juice was finally found to be about 1 litre. Squash was prepared by using the recipe : Ou-tenga segments 2 kg; water 1 litre; sugar 1 kg; citric acid 30 g; benzoic acid 2.5 g; yellow colour 2 drops.

Method B : Extraction of juice was similar to the Method A. But in the preparation of squash the recipe followed was : Ou-tenga segments 2 kg; water 1 litre; sugar 800 g; citric acid 20 g; benzoic acid 2.5 g and colour 2 drops.

Method C : Five hundred ml of ou-tenga extract (pulp) was mixed with equal quantity of tap water, filtered through muslin cloth and following ingredients were added : Ou-tenga pulp 500 ml; water 500 ml; sugar 600 g; citric acid 10 g; potassium-meta-bisulphite 0.1% and salt 3 g.

The squashes so prepared were kept in glass bottles and stored for 60 days. Biochemical parameters (pH, TSS, titrable acidity, reducing sugars, non-reducing sugar and total sugars) of squash were determined by following standard procedures as per AOAC (1980) and Ranganna (1986). Organoleptic parameters such as colour, flavour, taste and astringency were evaluated on 7 point scoring method of Jakobsen (1949). Analysis for biochemical composition and organoleptic quality was done at 0 day, 30 days and 60 days of storage at room temperature (30-34^{0}C) and RH of 81 per cent. The data were statistically analysed by using completely randomised design of Panse and Sukhatme (1978) at 5 per cent level of significance with three replications.

Table 1 shows the biochemical parameters of ou-tenga squashes prepared by Methods A, B and C. It generally reflected the recipe according to the addition of sugar, citric acid and other ingredients. pH and acidity levels were found

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TABLE 1. BIO-CHEMICAL PARAMETERS OF OU-TENGA (*DILLENIA INDICA*) SQUASH AND ITS STORAGE EFFECT.

Treatments methods	Storage period (days) D_1 (0)	D_2 (30)	D_3 (60)	Mean	D_1 (0)	Storage period (days) D_2 (30)	D_3 (60)	Mean
		pH				**Titrable acidity %**		
A	1.37	2.12	2.24	1.91	0.65	0.52	0.49	0.55
B	2.30	2.42	2.56	2.43	0.51	0.85	0.77	0.71
C	2.61	2.74	2.88	2.74	0.72	0.65	0.55	0.64
Mean	2.09	2.43	2.56		0.63	0.67	0.60	
		S.Ed(±)	$CD_{0.05}$			S.Ed(±)	$CD_{0.05}$	
Method		0.057	0.118			0.010	0.021	
Stroge		0.057	0.118			0.010	0.021	
Method x Storage		0.069	NS			0.012	NS	
		TSS %				**Reducing sugars %**		
A	48.0	48.0	48.5	48.17	35.08	40.00	45.45	40.18
B	34.5	34.5	34.8	34.60	19.61	20.00	29.41	23.01
C	29.0	29.0	29.2	29.05	13.13	10.20	16.67	13.33
Mean	37.16	37.17	37.50		22.61	23.40	30.51	
	S.Ed(±)	$CD_{0.05}$				S.Ed(±)	$CD_{0.05}$	
Method	0.157	0.324				0.101	0.208	
Storage	0.157	0.324				0.101	0.208	
Method x Storage	0.189	NS				0.121	NS	
		Total sugars %				**Non-reducing sugars, %**		
A	48.48	52.83	55.05	55.12	13.46	12.83	9.60	11.96
B	35.24	37.08	38.38	36.90	25.63	17.08	9.07	17.26
C	26.54	29.59	31.15	29.09	23.42	19.39	14.48	19.10
Mean	36.75	39.83	41.53		20.84	16.43	11.05	
	S.Ed(±)	$CD_{0.05}$				S.Ed(±)	$CD_{0.05}$	
Method	0.098	0.202				0.100	0.207	
Storage	0.098	0.202				0.100	0.207	
Method x Storage	0.118	NS				0.121	NS	

NS = Non-significant

to be in the range required for squashes which contribute to optimum taste. Biochemical analysis of squash during storage showed an increasing trend in pH, TSS, total sugars and reducing sugars while it showed a decreasing trend in titrable acidity and non-reducing sugar (Table 1). After 60 days of storage Method A recorded pH value of 2.24, Method B, 2.56 and Method C, 2.88. This increase in pH may probably be due to loss of H^+ ion concentration as a result of decrease in acidity during storage. Acidity was found to be 0.49% in Method A, 0.77% in Method B and 0.55% in Method C after 60 days of storage. This decrease in acidity might be due to conversion of some amount of acids to sugar (Babsky et al 1986). Method A recorded 48.50%, Method B 34.80% and Method C 29.20% of TSS after 60 days of storage. This increase in TSS during storage may be attributed to the enzymatic hydrolysis of polysaccharides into sugars. Reducing sugars continued to increase while non-reducing sugar decreased in all the methods used for preparation of ou-tenga squash during storage. The rate of increase of reducing sugars was found to be 22.81%, 67.32% and 81.22% in Method A, Method B and Method C, respectively after 60 days of storage. This rise in reducing sugars and gradual fall in non-reducing sugars might be due to conversion of non-reducing, owing to the process of hydrolysis. After 60 days of storage total sugars of squash was recorded to be 55.05%, 38.38% and 31.15% in Method A, Method B and Method C, respectively. The rate of increase in total sugars varied from 8.40 to 17.79%. This increase in total sugars might be assigned to the hydrolysis of complex carbohydrates. Khurdiya and Roy (1985) also reported similar biochemical changes during storage of jamun nectar. Increased pH, TSS, reducing sugars and total sugars and decreased non-reducing sugar and acidity during extended storage were also reported (Masoodi et al. 1992).

Ou-tenga fruit is difficult to process due to its hard texture and presence of gummy substances (Saikia and Dutta 1995). When it is cooked in autoclave, flavouring components get lost. In the present study, while cooking, blanching water was remixed in the pulp (extract) under Method A and Method B. The retention of flavour was maximum in Method B (9.8±0.2) followed by Method A (9.4±0.6) (Table 2). But in the Method C, where blanching water was not used in the preparation of squash, flavour retention was found to be lowest (7.8±1.2) Colour, taste and astringency scores were also found to be better under Method A and Method B in comparison to Method C (Table 2).

TABLE 2. EFFECT OF EXTENDED STORAGE OF DILLENIA SQUASH ON ORGANOLEPTIC QUALITY (SCORE)

Treatments/ Methods	Storage period, days D_1 (0)	D_2 (30)	D_3 (60)	Mean	Storage period, days D_1 (0)	D_2 (30)	D_3 (60)	Mean
		Colour				**Taste**		
A	9.6 ± 0.4	9.2 ± 0.8	7.8 ± 1.2	8.87	9.4 ± 0.6	9.2 ± 0.8	7.4 ± 1.6	8.67
B	7.8 ± 1.2	7.4 ± 1.6	5.8 ± 1.2	7.00	8.2 ± 1.2	7.4 ± 2.0	5.4 ± 1.6	7.00
C	7.4 ± 1.6	7.0 ± 2.0	5.0 ± 1.2	6.47	7.4 ± 1.6	6.6 ± 2.4	4.2 ± 0.8	6.07
Mean	8.27	7.88	6.20		8.33	7.73	5.67	
		S.Ed(±)	$Cd_{0.05}$			S.Ed(±)	$CD_{0.05}$	
Method		0.033	0.140			0.054	0.230	
Storage		0.033	0.140			0.054	0.230	
Method x Storage		0.116	0.243			0.190	0.399	
		Flavour					**Astringency**	
A	9.4 ± 0.6	8.2 ± 0.1	6.6 ± 0.4	8.07	4.2 ± 0.8	4.6 ± 0.4	6.2 ± 0.8	5.00
B	9.8 ± 0.2	8.2 ± 0.8	5.8 ± 1.2	7.93	3.0 ± 2.0	3.4 ± 1.6	3.8 ± 1.2	3.40
C	7.8 ± 1.2	6.2 ± 0.8	5.4 ± 1.6	6.47	2.6 ± 0.4	3.4 ± 1.6	3.8 ± 1.2	3.27
Mean	9.00	7.53	5.93		3.27	3.80	4.60	
		S.Ed(±)	$CD_{0.05}$			S.Ed(±)	$CD_{0.05}$	
Method		0.043	0.185			0.040	0.172	
Storage		0.043	0.185			0.040	0.172	
Method x Storage		0.153	0.321			0.142	0.298	

Score scale : 10 excellent, 9 every good, 7 good, 5 medium, 3 poor, 1 very poor, 0 unacceptable

During storage, organoleptic quality of ou-tenga squashes had significantly changed from initial to 60 days of storage irrespective of the method of preparation. Method A recorded the highest score (7.8±1.2) followed by Method B (5.8±1.2) and Method C (5.0±1.2) for colour after 60 days of storage. Incidence of such colour change during storage might be due to the oxidation and amino-acid sugar interaction leading to the formation of brown pigments.

From the results of this study, it may be concluded that ou-tenga can be successfully utilized for preparation of squash. Method A was found to be the best from biochemical as well as organoleptic point of view. However, Method B can also be used.

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RESEARCH NOTE

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Effects of Concentration and Temperature on the Rheology of Mango Pulp

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The rheological properties of mango pulp *"Chausa"* variety extracted from fully riped mangoes were determined for the solid content ranging from 10 to 20^0 Brix. The consistency coefficient (K) showed exponential correlation with pulp concentration. The consistency coefficient followed the Arrhenius temperature relationship and the magnitudes of energy of activation were found in the range of 8.9 to 11.8 kJ/g mole. The rheological parameter, consistency coefficient, significantly changed with concentration and temperature. The consistency coefficient was correlated to concentration and absolute temperature by a simple mathematical relationship, which is useful for equipment and process design.

Keywords : Mango pulp, Rheological characteristics, Consistency coefficient, Flow behaviour index, Activation energy.

Mango (*Magnifera indica* L.) is a tropical fruit relished for its succulence, exotic flavour and delicious taste throughout the world. India is the leading mango growing country of the world. The unripe fruit because of its acidic taste is used for preparing chutneys, pickles, etc, while the ripe fruit is used for preparing squashes, jams, nectar, mango leather, etc (Kumbhar 1992). The pulp and slices are canned and intermediate products like mango pulp and concentrates are exported. Presently, one of the major needs within the mango processing industry is to utilize mango pulp by developing a variety of processed products.

Rheology is defined as the science of deformation and flow of matter. In order to estimate the heat transfer coefficient of processing units, it is necessary to know rheological properties of foods and their variations with temperature over a wide range of concentrations and other processing conditions. Viscosity is considered as an important property related to the quality of food products. The viscosity data is also essential for the design and evaluation of food processing equipments such as pumps, heat exchangers and evaporators (Dodeja et al. 1990). The power law model has been extensively used (Harper and El-Sahrigi 1965; Vitali and Rao 1984) to relate the shear rate with (v) with shear stress (ι) for these foods.

$\iota = Kv^n$

where K = the consistency index and n = the flow behaviour index.

Typical values of consistency coefficient and flow behaviour index are not found in the literature for wide concentration and temperature ranges. Therefore, present investigation was undertaken to study and model changes in rheological behaviour of mango pulp as a function of concentration and temperature.

Sample preparation : Fully ripe "*Chausa*" variety of mango was procured from the local market during the July/August harvest season and were washed in water, peeled manually and pulped in a pulper fitted with 40-mesh sieve (Narang Corporation, New Delhi). The pulp was heated at 93^{0}C for 2 min to inactivate the pectinase and other enzymes and air-cooled (Nath and Ranganna 1980). It was preserved with 1730 ppm potassium metabisulphite (1000 ppm SO_2) and stored in refrigerator till further use. The pulp was concentrated using a hot water bath (70^{0}C) to a concentration of 10, 12, 14, 16, 18 and 20^0 Brix. The total soluble solids (TSS) content was measured using Abbe refractometer. The prepared samples were sealed in glass bottles till further study.

Rheological analysis : Rheological properties were measured using Brookfield viscometer (Brookfield Eng. Inc Model DVIII). The mango pulp was filled into 500 ml beaker with 8.5 cm diameter to a height of 8.5 cm and brought to a desired temperature in a water bath (TC - 500 Brookfied Eng. Inc). The spindle (No. 18) was immersed in the beaker and allowed to reach thermal equilibrium with the sample for 2 min. Observations were taken for shear stress and viscosity at different shear rates (2-14) and temperatures (30-70^{0}C).

Rheological characteristics : The effect of different levels of concentrations on apparent viscosity and shear rate at constant temperature, 50^{0}C is presented in Fig. 1. It was observed that as the shear rate increased the apparent viscosity decreased signifying the inverse relation between the two at a particular temperature. Also when the temperature was increased the value of apparent viscosity decreased but the plot trend remained the same at a particular temperature.

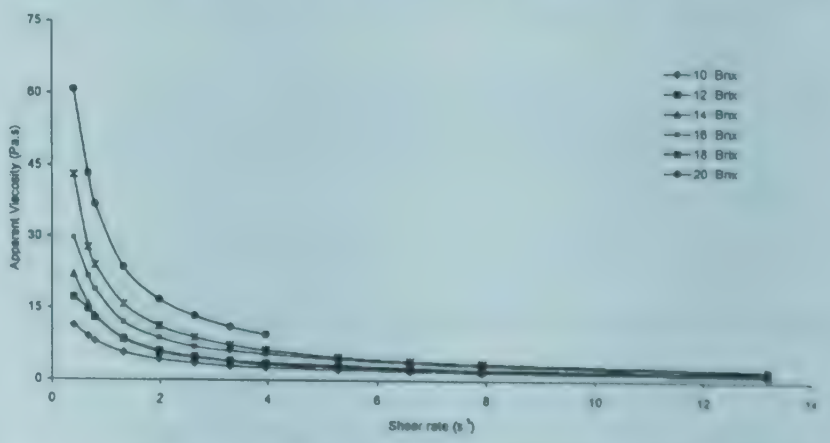

Fig. 1. Relationship between apparent viscosity and shear rate at 50^{0}C for different concentrations of mango pulp

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TABLE 1. CONSISTENCY COEFFICIENT, K (DYNE/CM^2.S) AND FLOW BEHAVIOUR INDEX , N OF MANGO PULP AS A FUNCTION OF CONCENTRATION AND TEMPERATURE.

Concn	10^0 Brix	12^0 Brix	14^0 Brix	16^0 Brix	18^0 Brix	20^0 Brix	
Temp							
30^0C	90.754	130.25	143.35	167.87	263.56	318.53	K
	0.2346	0.2531	0.1535	0.2190	0.1503	0.1638	n
	0.9864	0.9766	0.9156	0.9812	0.9813	0.9617	R^2
40^0C	70.275	106.38	127.77	155.26	223.88	304.07	K
	0.3192	0.2785	0.1967	0.2347	0.2062	0.1674	n
	0.9796	0.9770	0.9903	0.9905	0.9799	0.9765	R^2
50^0C	65.962	100.46	105.41	150.97	197.48	296.43	K
	0.3176	0.2843	0.1775	0.2374	0.1532	0.1730	n
	0.9907	0.9646	0.9619	0.9854	0.9949	0.9766	R^2
60^0C	60.797	94.275	101.30	109.70	182.31	284.39	K
	0.4174	0.2975	0.2241	0.3052	0.2268	0.1955	n
	0.9945	0.9662	0.9698	0.9817	0.9897	0.9862	R^2
70^0C	57.853	77.224	85.884	100.36	175.00	274.10	K
	0.4054	0.3174	0.1628	0.3055	0.2100	0.1918	n
	0.9840	0.9782	0.9637	0.9750	0.9827	0.9719	R^2

TABLE 2. CONSTANTS FOR THE EXPONENTIAL RELATIONSHIP BETWEEN TEMPERATURE AND MANGO PULP CONCENTRATION IN THE RANGE OF 10 TO 20^0BRIX

Temperature, ^{0}C	A	b	R^2
30	27.162	0.1221	0.97
40	18.191	0.1393	0.99
50	16.232	0.1414	0.97
60	15.033	0.1396	0.92
70	11.959	0.1484	0.93

The values of consistency coefficient (K) and flow behaviour index (n) of mango pulp as a function of concentration and temperature are shown in Table 1. The table shows that value of K increases with increase in concentration, whereas it shows a reverse trend with increase in temperature.

Effect of concentration : To visualize the influence of solid content on rheological behaviour of pulp, the plots of apparent viscosity as a function of concentration at each temperature were analyzed. The samples showed non-Newtonian behaviour. The rheological parameter, consistency coefficient, was related to the concentration by single term exponential model.

$$K = A \exp (bx) \quad \ldots\ldots\ldots \quad (1)$$

The model satisfactorily (R^2=0.92) fits the trend. The resulting magnitudes of constants A and b corresponding to exponential model are included in Table 2. As expected the consistency coefficient of concentrated mango pulp increased with its solid content. This exponential pattern for consistency has been reported for other food concentrates such as guava puree (Vitali and Rao 1982), tomato concentrates (Rao and Cooley 1983; Rao et al. 1981), orange juice (Hernandez et al. 1995), reconstituted milk (Reddy and Dutta 1994) and concentrated whole milk (Velez-Ruiz and Barbosa-Canovas 1996).

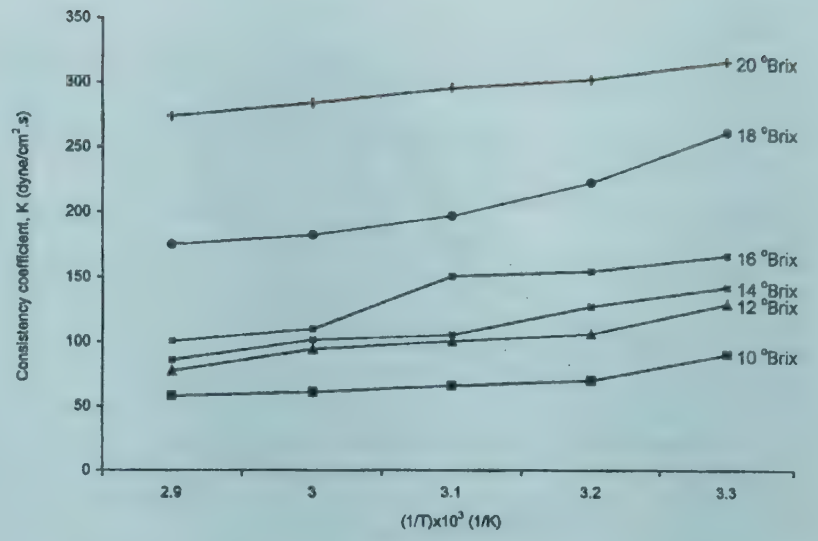

Fig. 2. Arrhenius curves for mango pulp

Effect of temperature : The effect of temperature on consistency of mango pulp followed Arrhenius equation where R^2 values were greater than 0.89.

$$K = K_0 \exp (E_a/RT) \quad \ldots\ldots\ldots \quad (2)$$

The Arrhenius curves for the mango pulps are shown in Fig. 2. The temperature has been reported to have a marked decreasing effect on apparent viscosity of fruit juices and this effect diminishes with increasing solid content. The consistency index decreased as the temperature increased while flow behaviour index was not affected much. The magnitudes of energy of activation were found in the range from 8.9 to 11.8 kJ/g mole. The low activation energy of mango pulp was similar to those obtained by Harper and El-Sahrigi (1965) on tomato concentrates.

Effect of concentration and temperature on mango pulp consistency : The effect of both variables on mango pulp consistency can be combined into a single logarithmic or exponential model (Velez-Ruiz and Barbosa-Canovas 1997) corresponding to Eq. (3) and (4) for simulation purposes.

$$\ln K = \beta_0 + \beta_1 (1/T) + \beta_2 X \quad \ldots\ldots\ldots \quad (3)$$

$$K = K_0 \exp (E_a/RT + \beta X) \quad \ldots\ldots\ldots \quad (4)$$

The natural log was employed on the consistency coefficient, the mango pulp concentration (^{0}Brix) and absolute temperature. The magnitudes of β coefficients corresponding to equations (3) and (4) were estimated by multiple linear regression (R^2=0.95). The effect of these two variables is opposite because solid concentration causes an increase in consistency coefficient and temperature diminishes it. The analysis of variance also revealed the significant effect of concentration and temperature on consistency coefficient.

Fig. 3 shows the three dimensional behaviour of consistency coefficient. One can determine the consistency coefficient of the pulp at a given concentration (X) and temperature (T) from either of the two relations expressed by the following equations.

$$\ln K = 2.3684 + 21.409/T + 0.13817\,X \quad \ldots\ldots\ldots \quad (5)$$

$$K = 10.68 \exp (^{21.409/T + 0.13817X}) \quad \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

Consequently, the proposed functional relationships for consistency coefficient as a function of concentration and

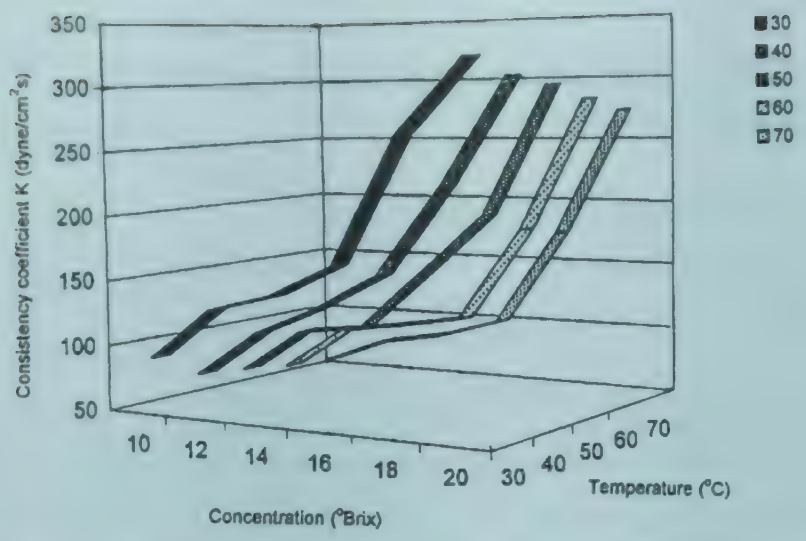

Fig. 3 Consistency coefficient as a function of concentration and temperature

temperature may be used for engineering and process simulation and design.

In conclusion, the mango pulp behaved as a non-Newtonian fluid. The one term exponential equation effectively defined the influence of mango pulp concentration on consistency coefficient where as Arrhenius model successfully showed the influence of temperature on consistency coefficient of concentrated mango pulp. The two-term model that combines concentration and temperature effects can be used in unit operation applications involving heat and mass transfer. Statistical analysis (ANOVA) indicated that rheological parameter (consistency coefficient) was highly influenced by concentration and temperature.

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RESEARCH NOTE

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Effect of Filleting and Marination Processes on the Quality of Roasted Chicken Breast Fillets

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Physico-chemical and sensory qualities of oven-roasted breast fillets (*Pectoralis superficialis*) harvested from hot (unchilled ≤ 1 h post-mortem), chilled (slush-ice; 2 h) or aged (4^0C, 24 h) broiler carcasses and either still-marinated or marinated in a tumbler (10 rpm; 15 or 30 min) were investigated. One group of fillets was mechanically (blade) tenderized before still marination. Data revealed that chilled or aged filleting produced a tender and juicier product with higher marinade uptake and lower cooking loss than hot filleting. Marinade uptake was highest in mechanically tenderized fillets (13.8%), followed by tumbled (10.0 - 12.9%) and intact muscle pieces (7.3%). Tumbling compared favourably with 2 h of still marination of blade-tenderized fillets in terms of marinade pick-up, cook-yield, shear value and sensory quality of the product. These results suggested that chilled filleting and rotary marination for 15 min would yield superior quality fillets with a greatly shortened processing time.

Key words : Deboning time, Broiler fillets, Still/rotary marination, Physico-chemical/sensory quality

Phenomenal increase in broiler production coupled with mechanized poultry processing, rapid urbanization and proliferation of fast food outlets have resulted in a steady increase in the demand for value-added poultry products in our country. This calls for development of innovative technology for newer products to offer variety and convenience to the consumers. Chicken breast fillets (*Pectoralis superficialis*) being low in fat and rich in protein may command consumer's preference in further-processed product segment of the Indian meat industry. Although some reports on the chicken breast filleting machine (Anon 1990) and marination techniques (Heath and Owens 1991; Xiong and Kupski 1999) are available, no such study seems to have been conducted in our country. Meat processors in order to reduce processing time and save energy adopt the practice of early harvesting of meat from unchilled carcasses. This, however, often results in increased meat toughness (Sams and Janky 1986). Hence, the present study was undertaken to evaluate the effect of hot, chilled and aged filleting and that of still marination with or without mechanical tenderization and tumbling on the physico-chemical and sensory quality characteristics of roasted broiler breast fillets.

Twentyone 6-week old broilers, reared under identical feeding and managemental conditions, were procured from the experimental farm of this institute, slaughtered conventionally and divided equally into three groups. Both left and right breast fillets from one group of warm (unchilled) eviscerated carcasses were removed within 1 h post-mortem by deskinning and severing the attachment of pectoral muscle from the shoulder joint and pulling it downward to strip the muscle from the carcass. Fillets from the other groups were harvested after chilling the carcasses in slush-ice (1 : 1.5 w/v) for 2 h or after aging the carcasses for 24 h in a refrigerator (4 ± 1^0C). The time interval between bleeding and filleting was so adjusted that both hot and cold deboned fillets could be simultaneously processed into marinated product by still marination (2 h). In order to evaluate the effects of still and rotary marination, a second lot of fillets harvested from chilled carcasses were still (unagitated) marinated as such for 2 h or after mechanical tenderization by passing fillets through a blade tenderizer (Hobart). For rotary marination, the intact fillets were tumbled at 10 rpm in a meat tumbler for 15 or 30 min. Ten fillets were assigned to each of the marination treatments.

The marinade consisted of 10% cereal starch, 2.5% common salt, 3% each spice mix and garlic-ginger (1 : 1) paste, 1% dextrose, 0.5% sodium tripolyphosphate, 0.4% citric acid and 0.02% tartrazine orange (permitted food colour). Fillets were individually identified by piercing numbered wing-bands through caudal end of the muscle, weighed, marinated (1 : 1 w/v) for specified time, drained for 15 min and reweighed to determine percent marinade uptake. Marinated fillets were then cooked in a forced draft oven set at 170^0C for 20 min after smearing the muscle surface with refined vegetable oil (60 ml/kg fillets), cooled to ambient temperature and reweighed to determine cooking loss as a percent of marinated weight. Five replications per treatment were used for determining percent marinade uptake and cooking loss.

Ten gram samples of cooked fillets were homogenized with 100 ml distilled water and the pH of slurry was determined in triplicates, using a digital pH meter. Standard AOAC (1980) methods were followed for the estimation of moisture, crude protein and ether extract contents of the product. Triplicate samples (3 x 1.5 x 0.6 cm) were cut parallel to the direction of muscle fibres from the middle part of cooked fillets from each treatment and sheared perpendicular to the direction of muscle fibres, using a Warner-Bratzler shear press. The product was evaluated organoleptically for appearance, texture, juiciness, flavour and overall acceptability on a 7-point Hedonic scale (7 = highly desirable; 1 = highly undesirable) by at least five experienced sensory panel members of this institute. Data were subjected to analysis of variance and significance between treatment means was determined by Duncan's multiple range test (Snedecor and Cochran 1968).

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TABLE 1. EFFECT OF HOT AND COLD FILLETING ON THE QUALITY OF MARINATED CHICKEN BREAST FILLETS

Characteristics	Hot (unchilled) (≤ 60 min post-mortem)	Chilled (slush-ice; 2 h)	Aged (4^0C; 24 h)
Marinade uptake, %	$6.54^a \pm 0.56$	$8.76^b \pm 0.51$	$9.06^b \pm 0.30$
Cooking loss, %	$24.85^a \pm 0.44$	$21.71^b \pm 0.41$	$20.97^b \pm 0.35$
pH	$5.96^a \pm 0.03$	$5.80^b \pm 0.02$	$5.73^b \pm 0.04$
Shear force, lb	$8.2^a \pm 0.30$	$6.4^b \pm 0.35$	$5.9^b \pm 0.26$
Sensory scores			
Appearance	6.2 ± 0.37	6.0 ± 0.32	6.2 ± 0.20
Texture	5.4 ± 0.24	6.2 ± 0.37	6.4 ± 0.40
Juiciness	$5.0^{a*} \pm 0.32$	$6.0^b \pm 0.32$	$6.4^b \pm 0.24$
Flavour	5.4 ± 0.24	6.2 ± 0.37	6.0 ± 0.32
Overall acceptability	5.2 ± 0.49	$6.2 \pm 0.3.7$	6.4 ± 0.24

Means bearning similar superscript or no superscript did not differ significantly ($P < 0.01$; $^*P < 0.05$)

The weight of raw fillets (*Pectoralis superficialis*) from each half of the broiler breast averaged 79.5 g and the paired fillets constituted nearly half (49.5%) of the breast part and 14.2% of the eviscerated carcass weight.

Hot/cold filleting : The effects of hot, chilled and aged filleting on some selected physico-chemical and sensory quality characteristics of marinated roasted breast fillets are presented in Table 1. The percent marinate uptake was significantly less in hot-deboned fillets than in fillets harvested after chilling or aging of carcasses. However, the same did not differ significantly between chilling and aging treatments, indicating that longer aging period had no beneficial effects on marinade pick-up. Hot deboned fillets not only picked up less marinade but also registered significantly ($P < 0.01$) higher cooking loss by about 3.1 and 3.8%, respectively as compared to chill-deboned and aged-deboned fillets. The pH and shear values of the product from these deboning treatments showed a pattern similar to that of cooking loss. These results indicated that hot-deboned fillets might be in the rigor state as evident from higher pH, cook loss and shear value. Kijowski et al (1982) have reported that the onset of rigor in chicken breast meat commenced within 30 to 60 min post-mortem. Although early and delayed filleting produced no statistically significant differences in sensory properties, except juiciness, of marinated fillets, the product prepared from chilled and aged carcasses received higher scores for texture, juiciness, flavour and overall acceptability than that from unchilled carcasses. Tenderness response in terms of texture and juiciness was closely related to shear value of the product.

Marination process : Of the marination techniques employed, both tumbling and still (unagitated) marination of mechanically tenderized fillets appeared superior to still marination of intact (non-tenderized) breast muscles in terms of higher marinade pick-up and cook yields, lower shear values and superior organoleptic quality of fillets (Table 2). The marinade uptake was found to be highest (13.8%) in still marinated blade tenderized fillets, lowest (7.3%) in similarly treated intact fillets and was intermediate (10.0 - 12.9%) in fillets subjected to tumbling marination. The multiple perforations in mechanically tenderized fillets produced by blade tenderizer leading to increased surface area for the absorption and adsorption of marinade might be responsible for this difference. Heath and Owens (1991) found that adsorption of marinade was a greater factor than absorption of marinade for variations observed in marinade uptake and the same was directly related to muscle surface area. The superior quality exhibited by tumbled intact fillets over non-tumbled intact fillets might be due to the extraction of myofibrillar proteins to the surface of meat on account of disruption of some myofibrils by the mechanical action of tumbler (Xiong and Kupski 1999). The binding ability of extracted proteins facilitated marinade pick-up during tumbling, possibly due to increased adhesion of marinade on the muscle surface. Furthermore, coagulation

TABLE 2. EFFECT OF STILL AND ROTARY MARINATION ON THE QUALITY OF MARINATED CHICKEN BREAST FILLETS

Characteristics	Still marination (2 h)		Tumbling (10 rpm)	
	Intact fillets	Blade tenderized fillets	15 min	30 min
Marinade uptake, %	$7.31^a \pm 0.30$	$13.78^b \pm 0.37$	$10.04^c \pm 0.27$	$12.86^b \pm 0.21$
Cooking loss, %	$22.50^a \pm 0.37$	$19.85^b \pm 0.33$	$17.72^c \pm 0.47$	$18.68^{bc} \pm 0.34$
pH	$5.92^a \pm 0.04$	$5.57^b \pm 0.03$	$5.49^b \pm 0.03$	$5.42^b \pm 0.02$
Shear force, lb	$8.2^a \pm 0.15$	$5.6^b \pm 0.10$	$5.1^b \pm 0.19$	$5.3^b \pm 0.09$
Moisture, %	$63.05^a \pm 0.38$	$65.03^b \pm 0.18$	$65.31^b \pm 0.33$	$65.28^b \pm 0.12$
Crude proteins, %	$30.16^a \pm 0.20$	$28.38^b \pm 0.13$	$27.56^b \pm 0.32$	$28.02^b \pm 0.28$
Ether extract, %	4.86 ± 0.04	4.46 ± 0.09	4.56 ± 0.08	4.78 ± 0.16
Sensory scores				
Appearance	5.8 ± 0.37	6.0 ± 0.32	6.2 ± 0.37	6.2 ± 0.20
Texture	6.0 ± 0.55	6.6 ± 0.24	6.4 ± 0.24	6.4 ± 0.40
Juiciness	$5.4^{a*} \pm 0.40$	$6.4^b \pm 0.24$	$6.6^b \pm 0.24$	$6.6^b \pm 0.40$
Flavour	5.8 ± 0.49	6.2 ± 0.37	6.2 ± 0.20	6.4 ± 0.24
Overall acceptability	5.8 ± 0.37	6.4 ± 0.40	6.2 ± 0.37	6.4 ± 0.24

Means bearing similar superscript or no superscript did not differ significantly ($P < 0.01$; $^*P < 0.05$)

of extracted muscle proteins might have acted as a sealing agent to facilitate moisture retention during cooking as evident from significantly higher moisture content in tumbled samples.

Incorporation of sodium tripolyphosphate in the marinade might have exerted a synergistic effect in improving the extractability of myofibrillar proteins during tumbling process (Froning and Sackett 1985) and associated beneficial effect on fillet quality. Reduction in cooking loss and/or shear values of chicken parts and turkey breast muscle due to tumbling was also demonstrated by Chen (1982) and Auday and Froning (1987), respectively. A significant decrease observed in pH of tumbled and mechanically tenderized fillets could be accounted for by more uptake and retention of marinade containing citric acid in these treatments. The crude protein contents differed significantly whereas ether extract varied within a narrow range (4.5 - 4.9%) between treatments.

Rotary marinated fillets had better appearance than still marinated fillets possibly due to uniformity of cooked surface and colour intensity. On the other hand, mechanically tenderized fillets were preferred for relatively softer texture than other treatments. Lower juiciness, flavour and overall acceptability scores of still marinated intact fillets were reflected by less marinade uptake and higher cooking loss and shear value.

Conclusively, it may be stated that slush-ice chilling of broiler carcasses for 2 h prior to filleting and tumbling (10 rpm; 15 min) marination could yield delicious marinated chicken breast fillets with added advantage of shortened processing time. Alternatively, blade tenderization of fillets prior to still marination would yield product of comparable quality.

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RESEARCH NOTE

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Effect of Combination of Improvers on Firmness of Bread from Few Punjab Wheat Varieties During Storage

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Effect of combination of improvers (potassium bromate and ascorbic acid) at 5 : 50, 10 : 100, 15 : 150 and 20 : 200 ppm respectively during storage (0, 24, 48, 72, 96 h) on firmness of bread from Punjab wheat varieties 'WH 542', 'PBW 343' and 'PBW 138' was investigated. Combination of improvers increased firmness of bread made from all the varieties. Firmness values increased as storage period increased. Three-factor interaction among combination of improvers, varieties and storage indicated that these factors were dependent on each other.

Keywords : Improvers, Storage, Bread firmness, Wheat varieties.

Improvers like potassium bromate and ascorbic acid have been used by some of the milling industries to improve the functional characteristics of wheat flour (Menon 1990). Singh et al (1979) reported that response of Punjab wheats to potassium bromate vary from negligible to very high, while Venkateshwara Rao et al (1986) found improvement with ascorbic acid in some Punjab wheats. Ravi and Haridas Rao (1995) reported that ascorbic brought about greater improvement in bread quality as compared to its combination with potassium bromate. A variety of factors contribute to firmness of bread including, product formulation (Marston and Short 1969; Short and Roberts 1971; Stutz et al. 1973; McDermott 1974; Tenny 1978) and process conditions (Waldt 1968; Kirk 1965; Kulp 1979). Short and Roberts (1971) and McDermott (1974) found that crumb firmness varied throughout the loaf, with the firmest at the center of the loaf. As information regarding response of combination of improvers and storage on firmness of bread from Punjab wheat varieties is not available, present experiment was carried out to study the above aspect.

Wheat varieties 'WH 542', 'PWB 343' and 'PWB 138' were obtained from 1996-97 harvest of Punjab Agricultural University, Ludhiana, India, conditioned to 14% moisture content for 24 h and milled using Brabender Quadrumat Junior Mill to get 70% extraction flour. Flour sample was stored in air tight Pearl Pet jars for further use. Fresh compressed yeast (Tower Brand) was obtained from the local market when required. Potassium bromate and ascorbic acid were obtained from Central Drug House (CDH), Mumbai, India.

Moisture, total ash, wet and dry gluten, Falling number (Perten), damaged starch and diastase activity were determined according to AACC (1990) procedures. SDS-sedimentation method of Axford et al (1979) was used to study the gluten strength.

Straight dough method (AACC 1990) was used in bread making. Breads were prepared after adding improvers (potassium bromate and ascorbic acid in solution form) on the flour weight basis. The ingredients i.e. flour (200 g), water (variable), fresh compressed yeast (6 g), salt (2 g), sugar (10 g) and improvers (variable) were mixed in laboratory mixer (National Manufacturing Co. Nebraska, Lincoln) for 1.5 min and fermented at 30^{0}C and 85% RH in the fermentation cabinet (National Manufacturing Co. Nebraska, Lincoln) for 1 hr 30 min. After punching, second fermentation was carried out for 30 min. Then the loaves were mechanically molded and proofed at 30^{0}C and 85% RH for 25 min. Baking time was 25 min at 220^{0}C in a rotary oven (National Manufacturing Co. Nebraska, Lincoln). The unsliced loaves were placed in polyethylene bags, coded and stored at 24^{0}C.

Two outside crust portions (approximately 6 cm at each end) were discarded, the loaves were sliced into 25 mm thick slices and used immediately for measuring firmness on Instron (Model 4464) Universal Testing Machine (Instron Corp., Canton, MA, USA). The rate of compression or crosshead speed was set at 100 mm/min. Flat aluminum plunger with a diameter of 36 mm (1017.9 mm^2), was used to compress the 25 mm thick slice to a 40% of total compression (10 mm compression depth) according to AACC (1990) procedure. The whole procedure was repeated on three different slices from the same sample and the firmness values were averaged. The bread firmness measurements were also carried out after 24, 48, 72 and 96 h of storage (Sidhu 1999).

The firmness data was analysed for statistical significance using three-way ANOVA according to Steel and Torrie (1986).

Chemical characteristics of wheat flour (Table 1) indicated that flour from wheat variety 'WH 542', 'PWB 343' and 'PBW 138' were medium type. The flour from all the varieties had low alpha amylase activity as revealed by high falling number.

Firrnness evaluation : The analysis of variance data (Table 2) for the effect of incorporating combination of improvers (potassium bromate : ascorbic acid; 5 : 50, 10 : 100, 15 : 150 and 20 : 200 ppm) on firmness of bread from different wheat varieties 'WH 542', 'PWB 343' and 'PWB 138' showed a significant variety, storage and combination effect. The various two factor interactions were also significant, indicating that the effect of storage period and combination of improvers

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TABLE 1. QUALITY CHARACTERISTICS* OF FLOUR FROM DIFFERENT WHEAT VARIETIES (n = 3)

Characteristics	'WH 542'	'PBW 343'	'PBW 138'
Extraction rate, %	70.00	69.00	69.00
Ash, %	0.58	0.62	0.48
Gluten (wet), %	30.00	25.50	24.94
Gluten (dry), %	11.20	9.00	8.75
Falling number, s	712.00	533.00	748.00
SDS-Sedimentation value, ml	34.00	29.00	26.00
Diastatic activity, mg/10 g flour	112.00	234.00	112.00
Damaged starch, %	5.30	4.20	6.60

Expressed on 14% moisture basis

TABLE 2. MEAN SQUARE VALUES FOR THE EFFECT OF INCORPORATING COMBINATION OF IMPROVERS; POTASSIUM BROMATE AND ASCORBIC ACID (5 : 50, 10 : 100, 15 : 150, 20 : 200) ON THE FIRMNESS OF BREAD FROM 'WH 542', 'PBW 343', 'PBW 138'

Main effect	df	MSS combination
A	2	23891536*
B	4	6600488*
C	4	3039392*
(A x B)	8	820112*
(A x C)	8	1042612*
(B x C)	16	138026*
(A x B x C)	32	139935*
Error	75	

MSS-Mean sum of squares, A - Varieties, B - Storage time, C - Combination of improvers

*$p < 0.05$

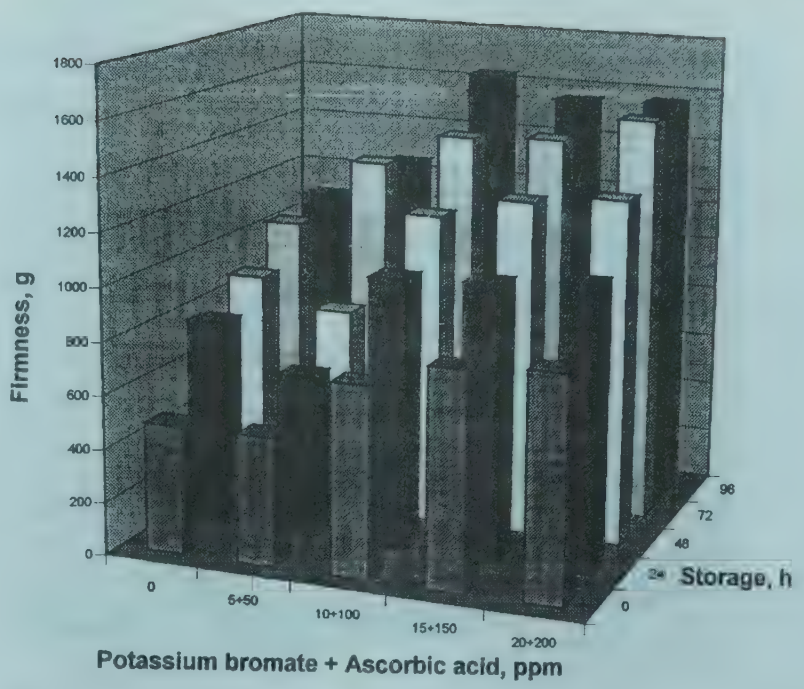

Fig 1. Effect of combination of improvers and storage on bread firmness from 'WH 542'

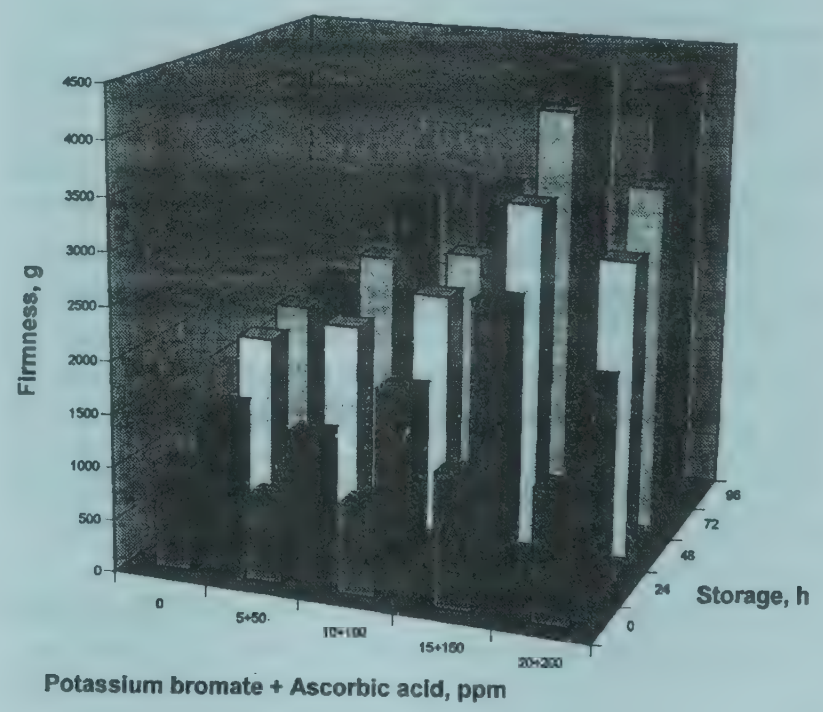

Fig 2. Effect of combination of improvers and storage on bread firmness from 'PBW 343'

were dependent on the variety. A significant three factor interaction indicated that these factors were dependent on each other. Increase in firmness was due to movement of moisture from gluten to starch by diffusion, which is responsible for staling (Hodge 1977). Ascorbic acid acts as an intermediary in oxidation process in dough, first being oxidized by incorporated oxygen during mixing to dehydro-ascorbic acid (DHAA) which in turn reacts with the thiols of gluten protein to form disulphide, causing marked strengthening of dough. Combination of improvers seem to fail in preventing the movement of moisture from gluten to starch by diffusion in these wheat varieties. Further, ascorbic acid acted fast as an oxidizing agent, toughened the dough, which already had weak gluten, thereby impairing the baking and firmness properties of bread.

Combination of improvers resulted in an increase in firmness values (Fig 1,2 and 3) from all the varieties. Bread firmness was maximum at 10 : 100 ppm combination of potassium bromate and ascorbic acid, after 96 h of storage for 'WH 542', at 15 : 150 ppm for 'PBW 343' and at 20 : 200 ppm for 'PBW 138' respectively. This may be attributed to the action of ascorbic acid, being an electron donor, it contributes firmness to the protein structure (Nakamura and Kurata 1997). Wheat variety having more gluten content ('WH 542') gave the firmest bread at less level of ascorbic acid in the combination as compared to those ('PBW 343', 'PBW 138'), with less gluten content, which gave the firmest bread at higher level of ascorbic acid in the combination. With the increase in storage, firmness increased considerably as compared to control. Combination of improvers did not improve the quality of bread from these Punjab wheat varieties with respect to firmness.

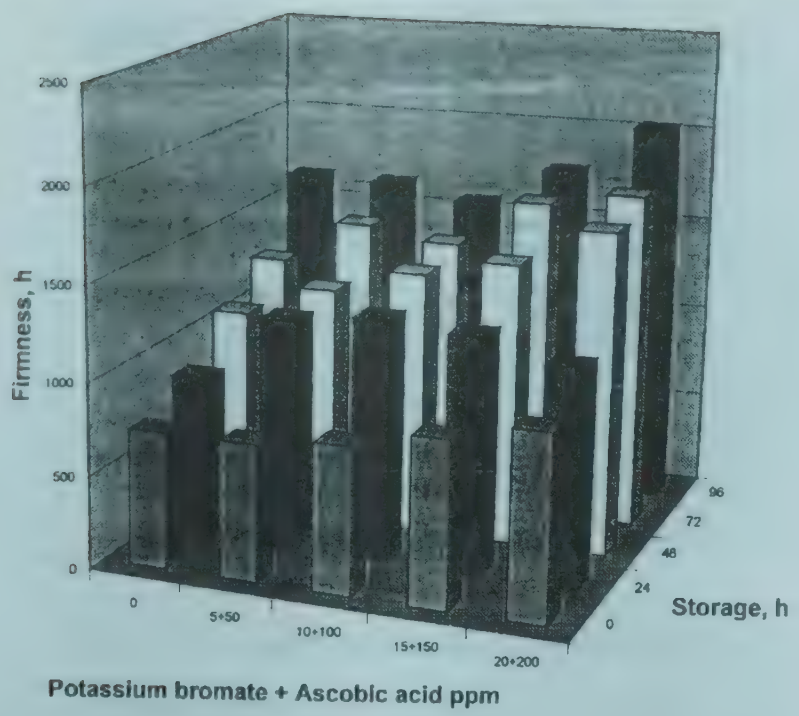

Fig 3. Effect of combination of improvers and storage on bread firmness from 'PBW 138'

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RESEARCH NOTE

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Effect of Different Thermal Treatments on Vitamin C and Microbial Sterility of Canned Drumstick (*Moringa oleifera*)

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Drumstick (*Moringa oleifera*), a low acid vegetable known for it's high vitamin C content has export potential as a canned product. It retains its wholesomeness and quality in terms of vitamin C retention after canning. Retention of vitamin C depends on the parameters of time temperature combination of canning, which is presented in this investigation on drumsticks. Further loss of vitamin C was determined at three retorting temperature at 110^{0}C, 115^{0}C and 121^{0}C, respectively. It was found that required retorting time for sufficient lethality at 115^{0}C and 121^{0}C were 27 and 20 min. respectively. However, processing at 110^{0}C for 32 min, which was the total time for lethality calculations together with come up time was not sufficient to reach the required rate of lethality. In addition, microbial counts and incubation tests were also carried out to investigate for commercial sterility. The results indicated that vitamin C content in the fresh sample of the *Moringa oleifera* was 118 mg/100g edible portion. Loss of vitamin C after canning at 110^{0}C, 115^{0}C and 121^{0}C were 42%, 37% and 31% respectively suggesting that the best combination is 20 min at 121^{0}C. Incubation test showed that this treatment did not support the growth of aerobic or anaerobic microbes and there was no spoilage.

Keywords : Drumstick, *Moringa oleifera*, Canning, Time temperature combinations, Lethality rate, Loss of vitamin C, Microbial sterility.

Vitamin C is required in the diet in greater amounts than all other vitamins combined (FAO/WHO 1974). Fennema (1982) has shown that factors that can influence the nature of vitamin C degradation mechanism include temperature, salt, sugar concentration, pH, oxygen, enzymes and metal catalysts. Kailaspathy and Koneshan (1986) have shown that, among the popular green leaves consumed in Sri Lanka, the leaves of *Moringa oleifera* has the highest content of vitamin C (86 mg/ 100 g). Loss of vitamin C was found to an extent of 74% when stored for 24 h at 4.4^{0}C. Lubaza (1972) has shown that loss of 15-20% of vitamin C from refrigerated vegetables, subsequently became unacceptable as evidenced by a sensory evaluation point of view. The effect of wilting under low and moderate temperatures on the loss of vitamin C in vegetables was reported by Ezell and Wilcox (1959). Kailasapathy and Koneshan (1986) also showed that wilting of the freshly harvested leafy vegetables lost their ascorbate content by 58% after 12 h storage at room temperature. Fennema (1982) reported that retention of vitamin C content in green peas after freezing, canning and cooking were 71% 63% and 44%, respectively. Okeibuno (1991) investigated the retention of vitamin C in Nigerian green leaves that was 67% with steam blanching and 42% with water blanching.

For preserving, vegetable canning is being popularized in Sri Lanka particularly for export purpose. In general, retaining product quality with reference to microbial sterility by incubation tests, seemed preferable to nutritional quality. However, vitamin C is abundant nutrient present in some of the fruits and vegetables and is unstable at pH above 5.0 (Oliver 1981). Therefore, very often, vitamin C retention is considered as a quality parameter in low acid canned foods. Nevertheless, losses of vitamin C in low acid canned foods have not been fully investigated in Sri Lanka. In this study, heat treatments, at 110^{0}C, 115^{0}C and 121^{0}C has been employed to determine the retention of vitamin C. In addition, incubation tests, microbial sterility and product analysis have also been included. Therefore, this work was undertaken to determine the precise time temperature combinations for canning of drumstick (*Moringa oleifera*) and retention of vitamin C.

Freshly harvested fruits of *Moringa oleifera* were used in the study. Fifty grams of fresh pods were crushed in 100 ml of distilled water and pH of the resulting extract was measured by a glass electrode pH meter. In order to reduce the microbial load, unpeeled fruits were washed in 10 ppm chlorine and then hand peeling was done to remove their fibrous coat, cut into 12 cm pieces, rinsed in potable water and 235 g of pieces longitudinally packed into each can (plain A2) containing 195 ml of 2.5% brine, this resulted in 55% solid content. Leaving the standard headspace, double seeming was done to ensure adequate sealing. Sample lots of five cans were taken for test and each time-temperature combination was done in triplicate.

Lethality rate : The rate at which heat is conveyed into the cans was measured using thermocouples connected to a digital thermometer (Fluke 2190 A). The cold point of the cans was taken as it's geometric center. Reference to the pathogenic organism *Clostridium botulinum*, heat penetration data and the lethality rate were calculated according to the method described by Patashink (1953) as given below. Using this data, process times were calculated for three different temperatures; 110^{0}C, 115^{0}C and 121^{0}C.

$$\text{Lethlity rate} = \frac{1}{\log^{-1}\left(\frac{250 - t}{z}\right)}$$

Process time = Lethality time + come up time.

Vitamin C content was analyzed using dye reduction technique as described by Oliver (1981). In the presence of

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2-6 dichloro- indophenol dye, ascorbic acid is readily oxidized leading to colour change which was measured by a colorimeter (Sprectronic 21) at the 350 mm. Standard plot was done using known concentration of ascorbic acid. Of the 25 g. of vegetable crushed in 0.04% oxalic acid to stabilize the oxidation dye was added immediately before the reading was taken. For each set of samples biank determination was also carried out. Vitamin C content was calculated using the following formula.

$$\text{Vitamin C content mg/100g} = \frac{L_1 - L_2 \times V \times 100}{0.064 \times W \times dm}$$

Where, V =volume of the oxalic acid extraction; L_1 =colorimeter reading for the sample; L_2 =blank reading; W =weight of the sample; and dm =dry matter content of the sample. 0.064 = regression coefficient.

Apart from the determination of the remaining vitamin C content in the products which had been subjected to different thermal treatments, the vitamin C content of the unpeeled pods, peels and peeled flesh was also determined. Significant differences in the vitamin C contents of the treatments have been analyzed under randomized complete design.

Initially microbial screening was done at different stages i.e, before peeling, after peeling and after chlorination. The tests were carried out with 50 g. of fresh vegetables mixed with 100 ml of distilled water and serially diluted for three times to get the desired colonies on to aerobic plate counting. After processing, sample of 5 cans taken from each thermal treatment were subjected to incubation test at 37^{0}C for 14 days and inspected for swelling, as the commercial practice adopted in industries. For assessing the microbial sterility of the products, 5 ml of food sample taken from each can (which had been subjected to different thermal treatments) was spread on the nutrient agar medium and incubated at 37^{0}C for 3 days and inspected for the colonies. Using a similar sample, anaerobic incubation was done on a pork infusion agar medium and incubated in anaerobic jar at 42^{0}C for four days and finally microbial colonies were inspected. Canned products were analyzed for net weight, and drained weight using the weight difference. Total soluble solids was measured by a refractory indicator. Hand-held vacuum tester was used to measure the vacuum and the pressure was recorded in the gauge.

Chemical analysis revealed that unpeeled pods of *Moringa oleifera* had 124 mg. of vitamin C in 100 g. portion. Wickramanayaka (1987) had indicated that the pods of this vegetable contain 118.0 mg of vitamin C and the variability may be due to either variety or maturity differences. The vitamin C content in peeled pods and in the outer covering were 150 mg/100g and 70 mg/ 100 g respectively. Thus, peel which is discarded accounts for about 20% weight (wet basis) and the loss of vitamin C is about 14%. It was found that pH of the fresh drumstick pods was 5.2, confirming that it is a low acid vegetable. Come up times of the retort were found to have different values at different temperature when retorting at 110^{0}C, 115^{0}C and 121^{0}C, the respective come up times were 18, 11 and 10 min. This describes that to reach the standard canning temperature (121^{0}C) low acid foods require about 10 min.

Lethality rate : The calculated lethality rate from the Table 1 and the respective processing time from Table 2 shows that when retorting at 121^{0}C the required processing time was 20 min and for 115^{0}C the time requirement was 27 min. When retorting at 110^{0}C, it was found that the calculated lethality had not been sufficient (Table 1). This suggests that processing low acid foods at 110^{0}C even for 32 min will not assure the commercial sterility with reference to the *Clostridium botulinum* as the test organism.

TABLE 1. LETHALITY RATES AT DIFFERENT PROCESSING TEMPERATURES

	Retort temperature					
	110^0 C		115^0 C		121^0 C	
Time min	Mean can temp.^{0} C	Lethality rate	Mean can temp.^{0} C	Lethality rate	Mean can temp.^{0} C	Lethality rate
0	63.2	1.94×10^{-06}	62.1	8.9×10^{-07}	63.3	1.945×10^{-06}
2	70.9	1.23×10^{-05}	75.9	3.89×10^{-05}	71.2	1.23×10^{-05}
4	97.9	6.16×10^{-03}	99.1	7.76×10^{-03}	97.1	4.89×10^{-03}
6	100.9	0.0123	106.9	0.0489	106.9	0.0489
8	104.9	0.0309	117.7	0.1479	114.4	0.2818
10	106.1	0.0524	113.3	0.2440	118.8	0.7762
12	108.9	0.0776	114.4	0.2818	119.9	0.8120
14	109.8	0.0977	112.7	0.2454	117.3	0.5495
16	110.3	0.1096	108.9	0.0977	110.8	0.1230
18	106.5	0.676	89.6	9.67×10^{-04}	97.02	4.89×10^{-03}
20	73.1	1.26×10^{-05}	67.3	4.89×10^{-06}	73.5	1.25×10^{-05}
22	54.5	2.45×10^{-07}	51.4	1.23×10^{-07}	67.1	4.87×10^{-06}
24	35.6	3.08×10^{-09}	43.5	1.94×10^{-08}	63.2	1.94×10^{-06}

TABLE 2. PROCESSING TIME AT DIFFERENT TEMPERATURES

Steam pressure psi	Retort temperature ^{0}C	Come up time min	Lethality time min	Processing time min
7	110	18	ND	32
11	115	11	16	27
17	121	10	10	20

TABLE 3. LOSS OF VITAMIN C AT DIFFERENT THERMAL TREATMENTS

Temp. ^{0}C	Processed time. min	R_1	R_2	R_3	Avg. retention %	Avg. loss %
110	32	65	60	62	58	42
115	27	73	76	71	63	37
121	20	85	86	80	69	31

TABLE 4. BACTERIAL COLONIES

Step	Colonies/25ml	Colonies/g
Before peeling	13	2.6
Before peeling, Cl_2 treated	02	0.4
After hand peeling	06	1.2

Processing at 110^{0}C for 32 min caused a remarkable drop in vitamin C content than other thermal treatments. Processing at 115^{0}C and 121^{0}C accounted for 37% and 31% loss of vitamin C. Table 3 shows a clear relationship in that the greater the processing time, the greater the vitamin C loss. Heating at 121^{0}C is the common heat treatment for low acid foods and minimum loss of vitamin C was attributed in this experiment.

Hence, it is preferable to process *Moringa oleifera* at 121^{0}C for 20 min in order to conserve the vitamin C rather processing at low temperature for long time conditions. Processing at 115^{0}C ensured the microbial safety of the products but fairly high loss of vitamin C was experienced.

On the other hand processing at 110^{0}C has not assured the correct lethality of the product and it detrimentally affected the vitamin C content. Table 4 indicates that 13 aerobic colonies were present in the purchased product presumably due to contamination during transportation and handling. The 10 ppm chlorine treatment was shown to reduce the bacterial colonies to 2 after handling, further contamination seems to have raised the number of colonies.

However, incubation tests done with the canned products revealed that none of the cans produced under different thermal treatments showed signs of swelling and indicated microbial safety. Goldensberg et al (1985) have proposed that the satisfactory level of total counts in low acid canned foods be less than 10 colonies / g. But samples taken from the thermally processed canned drumsticks showed no single microbial colony either on aerobic or anaerobic plating. Therefore, under the studied conditions, the canned drumstick is safe and conform to the required standards regardless of the thermal treatments given. Product analysis carried out for the processed cans indicated that they complied to the quality standard required as a canned food and the results obtained were solid content 55%, vacuum 12.0 mm Hg, head space 4/16", total soluble solids 4.1 Brix, drain weight 290.0g and net weight 550.0 g.

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RESEARCH NOTE

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Effect of Sodium Acid Pyrophosphate and Sodium Lactate on the Viability of *Listeria monocytogenes* in Raw Buffalo Meat Mince

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The inhibitory effect of the sodium acid pyrophosphate (SAPP) and sodium lactate (SL) in raw buffalo meat mince stored at 4^0C for 16 days and 37^0C for 36 h against *Listeria monocytogenes* was carried out. Minced meat was evaluated for physico-chemical (pH, extract release volume) and microbiological (mesophiles, psychrophiles, *Listeria sp.*) qualities. Addition of SAPP (0.5%) to meat mince significantly ($P<0.05$) inhibited *L.monocytogenes* after day 10 of storage at 4^0C but favoured the growth of the pathogen at 37^0C. A mixture of SAPP (0.5%) and SL (2.5%) significantly ($P<0.05$) reduced the count of the pathogen at both the storage temperatures. This combination of food grade preservatives could be used as effective listeriostatic in minced meat stored at 4^0C as well as at 37^0C.

Keywords : Growth inhibition, *Listeria monocytogenes*, Sodium acid pyrophosphate, Sodium lactate, Buffalo meat mince.

Listeria monocytogenes is a food-borne pathogen capable to grow at refrigeration temperature (Walker et al. 1990), high salt concentrations (Shahamat et al. 1980) and relatively low pH (George et al. 1988). It is ubiquitous in nature (Brackett 1988) and has been isolated from variety of food items including raw milk (Bhilegaonkar et al. 1997), meat (Wang et al. 1992), and Vegetables (Arumugaswamy et al. 1994).

The anti-listerial effects of a range of food additives including polyphosphates (Sofos 1986), lactates, acetate (Houtsma et al. 1993) and parabens (Yousef et al. 1991) are well documented. Sodium acid pyrophosphates (SAPP) has been found to inhibit bacterial growth including that of *Clostridium sporogenes* strain 3679 inoculated in cooked, vacuum packaged bratwusrt held at 24-25^0C for 24 and 48 h (Molins et al. 1985). High molecular weight sodium polyphosphates in combination with sodium chloride have been suggested to be useful in controlling the growth of *L. monocytogenes*, especially at low temperature (Zaika and Kim 1993). Sodium lactate (SL) has been extensively used for prolonging shelf life of meat products especially cured meat products such as ham, bacon, cured beef and turkey salami at 1-7^0C (Houtsma et al. 1993).

The survivability of pathogenic *L. monocytogenes* against SAPP and SL in raw buffalo meat mince stored at 4^0C for 16 days and 37^0C for 36 h was studied and presented.

Pathogenic strain of *L. monocytogenes* MTCC 1143 (NCTC 11994) was procured from Institute of Microbial Technology, Chandigarh, India. The growth approximation of standard strain in bacterial suspension was done by McFerland Nephelometric technique as described by Paik and Suggs (1974). Subsequently, the bacterial growth quantified as per the method of ICMSF (1978) was adjusted so as to give counts of approximately 10^3 cells per 0.1 ml of the bacterial suspension.

The fresh raw buffalo meat purchased from retail outlets at Bareilly city, India was finely minced in sanitized meat mincers (Electrolux, Model 320064, Sweden) using 8 and 6 mm sieve. The samples each weighing 150 g were drawn from the minced meat in sterilized polyethylene sachets and analyzed for physico-chemical parameters like extract release volume (Shelef 1974), pH (AOAC 1975) and microbiological parameters like total viable count (TVC) for mesophiles and psychrophiles (ICMSF 1978), besides presumptive *L. monocytogenes* count so as to adjudge the initial microbial status of buffalo meat. For estimating mesophiles and psychrophiles plate count agar and for presumptive *L. monocytogenes* count Dominguez-Rodriguez isolation agar (DRIA; Dominguez-Rodriguez et al. 1984) were used.

Each of the meat sample contained in polyethylene sachets (150 g) was treated separately with a final concentration of 0.5% sodium acid pyrophosphate, 0.5% sodium acid pyrophosphate + 2.5% sodium lactate and control with no preservatives.

The meat mince samples were inoculated with the quantified and calibrated bacterial suspension at the rate of 10^3 bacterial cells per gram of meat. Sachets were sealed and contents were mixed in the stomacher (Model BA 6021, Seward Laboratory, London) for 3 min and then incubated at 4^0C and 37^0C for 16 days and 36 hours, respectively, for each group under study.

From control and treatment groups each, 10 g aliquots were drawn on days 1, 4, 7, 10, 13 and 16 stored at 4^0C and on hours 12, 24 and 36 from those incubated at 37^0C. Each aliquot was then processed for estimation of *L. monocytogenes* count using DRIA and estimation of pH value.

The data obtained were statistically analysed using randomised block design as described by Snedecor and Cochron (1968).

The total viable counts (TVC) of mesophiles and psychrophiles were $3.2x10^5$ and $1.4x10^5$ per gram of meat, respectively. No *Listeria* sp. was detected from raw meat samples before treatment with preservatives. The initial pH and ERV of meat were 5.75 and 48 ml, respectively. These parameters were within the acceptable limits as the normal

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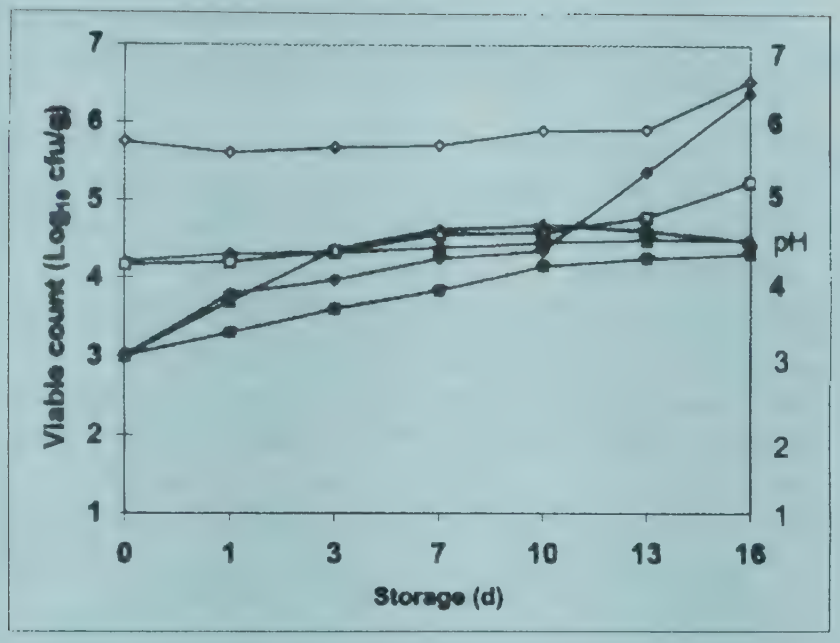

Fig. 1. Changes in the counts of *Listeria monocytogenes* and pH of control and treated raw buffalo meat mince during storage at 4^{0}C.

--◆--, Viable count in control; --◇--, pH changes in control; --■--, Viable count in treated sample with 0.5 % SAPP + 2.5 % sodium lactate; --□--, pH changes in treated sample with 0.5 % SAPP + 2.5 % sodium lactate; --▲--, Viable count in treated sample with 0.5 % SAPP; --Δ--, pH changes in treated sample with 0.5 % SAPP

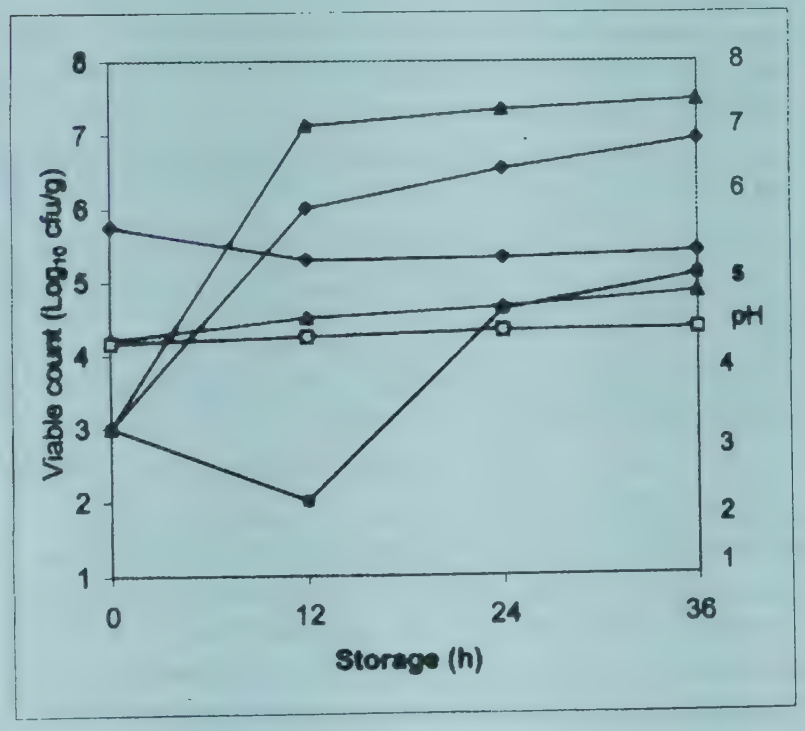

Fig. 2. Changes in the counts of *Listeria monocytogenes* and pH of control and treated raw buffalo meat mince during storage at 37^{0}C.

--◆--, Viable count in control; --◇--, pH changes in control; --■--, Viable count in treated sample with 0.5 % SAPP + 2.5 % sodium lactate; --□--, pH changes in treated sample with 0.5 % SAPP + 2.5 % sodium lactate; --▲--, Viable count in treated sample with 0.5 % SAPP; --Δ--, pH changes in treated sample with 0.5 % SAPP

values have been reported to be 5.8 for pH and 48-53 ml for ERV, less than 1.0×10^6 organisms per g for TVC of psychrophiles (Nickerson and Sinskey 1972) and 5.0×10^6 to 1.5×10^7 per g for TVC of mesophiles (Jay 1986).

In the control sample the count of *L. monocytogenes* increased consistently from an initial 3 $\log_{10}$ cfu/g to 6.38 $\log_{10}$ cfu/g on day 16 of storage at 4^{0}C. Addition of 0.5% SAPP in meat mince showed an initial reduction in the count of *L. monocytogenes* (Fig 1), afterwards it registered an upward trend in the count from 4 to 10 days and again showed a significant ($P < 0.05$) decrease on 13 - 16 days. On the contrary, incorporation of 0.5% SAPP in combination with 2.5% SL showed a significant ($P < 0.05$) inhibition from the day 1 in comparison to the control and the growth of the pathogen was suppressed by more than 2 $\log_{10}$ at the end of storage.

Addition of 2.5% SL along with 0.5% SAPP to meat inhibited the growth of *L. monocytogenes* at 4^{0}C on day 1 in comparison to the addition of 0.5% SAPP alone which inhibited the pathogen only at 10th day of storage. The observed inhibition of the pathogen by SAPP at 4^{0}C was in concurrence with the reports (Spencer and Smith 1962; Chen et al. 1973) where dipping of chicken parts or carcasses in phosphate containing chilled water have been reported to decrease the bacterial counts and prolong the shelf life.

Addition of SAPP to meat inhibited the pathogen immediately and thereafter its action was delayed from 4 to 10 days of storage at 4^{0}C which can be explained in the light of the findings of Molins et al 1985 who reported that the presence of enzymes, poly and pyro-phosphatases, in muscle cells might have accounted for the loss of the inhibitory properties of phosphate.

The pH of meat in the control sample increased consistently from an initial of 5.75 to 6.32 on 16 d of storage (Fig 1). Treatment of meat with 0.5% SAPP registered an initial increase in the pH by day 1 which further increased up to day 10 but decreased thereafter. Incorporation of 0.5% SAPP with 2.5% SL decreased pH of meat which remained less throughout the storage period in comparison to the control.

At 37^{0}C in control sample the count of *L. monocytogenes* increased rapidly from an initial 3 $\log_{10}$ cfu/g to 6.00 $\log_{10}$ cfu/g at 12 h and then gradually to 6.93 $\log_{10}$ cfu/g of meat at the end of storage (Fig 2). Addition of 0.5% SAPP in meat favoured the growth of *L. monocytogenes* as the count increased at 12 h and 36 h. On the contrary, a significant ($P<0.05$) reduction in the growth of the pathogen was noticed in meat treated with 0.5% SAPP with 2.5% SL throughout the storage period. The inhibition was maximum at 12 h than at 24 h and 36 h. Polyphosphates have been found to favour the growth of microorganisms in the processing and chill storage of poultry (Mead and Adams 1979).

AT 37^{0}C, pH in the control sample increased consistently from 12-36 h (Fig 2). Incorporation of 0.5% SAPP in combination with 2.5% SL showed a decline in pH throughout the storage period, whereas, addition of 0.5% SAPP showed an increase throughout the storage at 37^{0}C.

The listeriostatic effect exerted by SAPP was observed at 4^{0}C but not at 37^{0}C which was in accordance with the report (Zaika and Kim 1993).

A combination of 0.5 % SAPP with 2.5 % SL added in

meat significantly reduced the count of the pathogen at both the storage temperatures in comparison to the control, whereas, SAPP (0.5%) added alone reduced the count after 10 d of storage at 4^0C. Shelef and Yang (1991) also reported that lactate at 4 % level suppressed the growth of *L. monocytogenes* especially at lower temperature.

The pattern of inhibition of the pathogen after treatment with SAPP (0.5%) and SL (2.5%) at both the temperatures was comparable with that observed by Motlagh et al (1991) who reported that lactate inhibited the growth of *Yersinia enterocolitica* for initial two weeks after which the growth was resumed and at 8 weeks the count was about 0.5 log less than the control.

The pH of meat was found to increase with increase in the count of *L. monocytogenes* at both the storage temperatures which is in agreement with that of Zaika and Kim (1993) who reported that inhibition of *L. monocytogenes* increased with decrease in temperature and was greater at pH 6 than at pH 7.

Results of this study indicated that the initial microbial status of the meat mince was within the normal limits and the combination of 2.5% SL along with 0.5% SAPP added to meat mince was found to significantly suppress the growth of *L. monocytogenes* at both the storage temperatures in comparison to the addition of 0.5% SAPP alone which was partly listeriostatic at 4^0C only.

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RESEARCH NOTE

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Gelation of Shear Treated Whey Protein Polymers/Aggregates

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The gelation of whey protein polymers/aggregates dispersions pre-sheared by high shear forces was investigated. Whey protein polymers/aggregates were obtained in a two-stage heating process. Samples were mixed for 5 min at 20,000 rpm and rheological properties were compared with non-sheared dispersions. Gels prepared from pre-sheared dispersions at pH 7.0 and 6.5 characterized higher storage moduli and lower phase angles. Pre-shearing of the dispersions obtained at pH 6.0 resulted in a weaker gel, probably because of higher aggregation. Non-sheared gels had a higher optical density than pre-sheared gels, which suggests formation of a gel composed of larger aggregates.

Keywords : Gelation, Polymerization, Whey proteins, Shear.

Whey protein gels find a wide range of applications in forming texture of a new product or mimic the texture of an existing one (Mleko 1997). Gelation is possible when attractive forces between the protein molecules are strong enough to overcome the repulsive forces. When the proteins are heated above their denaturation temperature they partially unfold which leads to the exposure of non-polar amino acids, and increased attraction between them. The exposure of reactive sulphydryl groups leads to the formation of inter- and intramolecular disulfide bonds.

According to the definition of a gel introduced by Ferry (1980) : "A gel is a substantially diluted system which exhibits no steady state flow". The question is what will happen if a gel matrix will be destroyed and a flow will occur? Will attractive forces between molecules, which were responsible for gelation cause the reformation of a gel matrix? Taking into consideration the strength of physical bonds, two types of gels can be formed : strong and weak. Weak gels are viscoelastic materials, which are more likely to be reversible (Mleko 2000 a). Under shear, the microstructure of a gel can be drastically modified and the rheological properties are then governed by structure breakdown and build-up (Yziquel et al. 1999).

Gelation of whey proteins was extensively studied (Mleko et al. 1994, 1997, Mleko 2000 b, Mleko and Gustaw 2000). At optimal conditions, it is possible to form weak gels at considerably low whey protein concentrations (Mleko and Foegeding 1999). Gels from whey protein dispersions preheated at pH 8.0 were obtained by decreasing pH to 7-6 (Mleko and Foegeding 2000). The pH induced weak gels obtained from pre-heated WPI dispersions were, under certain conditions, reversible (Mleko 2000 a). Hydrophobic interactions were probably responsible for the re-healing of the gel structure. Shearing conditions used in previous research were mild and re-healing of gel could be partially caused by re-junction of previous gel matrix fragments.

The aim of this work was to investigate the gelation of whey protein polymers/aggregates pre-sheared by high shear forces.

Two-step polymerization of whey proteins : Whey protein isolate (WPI) was obtained from DAVISCO Food Ingredients International (Le Sueur, MN). The protein content was determined by analyzing for nitrogen (macro Kjeldahl) and calculating the protein as N x 6.38 (AOAC 1984). WPI dispersion (3% w/w) was made by hydration in 0.1 M NaCl with constant mixing by a magnetic stirrer for 1 h at room temperature and adjusting the pH to 8.0 using 2 N NaOH. The WPI dispersion was heated in flasks for 50 min at 80^{0}C. Flasks were cooled in tap water to room temperature and the pH of the dispersion was adjusted to 6.0, 6.5 or 7.0 using 1 M HCl. These were further heated in glass flasks in a water bath at 80^{0}C for 1 h. After cooling the flasks in tap water the samples were placed in the rheometer. In another series of experiments, after cooling the samples were mixed for 5 min at 20,000 rpm using OMNI-MACRO ES homogenizer (Omni International Inc., Warrenton, VA), degassed for 2 h and then poured into the rheometer.

Rheological measurements : A Carri-Med CS 100 (Rheo, Great Britain) with a cone and plate geometry (6 cm, 4^0) was used. All experiments were conducted in an oscillation mode at a frequency of 0.05 Hz and a strain of 0.1. Gelation was monitored for 6 h at 21^{0}C.

Turbidity measurements : The turbidity (optical density at 630 nm) of gels after 6 h of gelation was measured with a Dynatech MR (Guernsey, Channel Islands).

Whey protein polymers/aggregates obtained by a two-stage heating were viscous gel/sol dispersions. Placed in the cap of rheometer formed a gel as phase angle decreased to about 11-14^0 (Fig. 1). The highest value of phase angle was noted for pH 6.0. At this pH the lowest phase angle was noted at the time zero. At this pH, closer to the isoelectric point of whey proteins, there is a stronger aggregation and a tendency to form aggregates and not a three-dimensional macro structure. Because of higher aggregation at this pH on the beginning a low value of phase angle was noted (Fig. 1). The highest value of the storage modulus was observed for a gel obtained at pH 6.5 (Fig. 2). This pH seems to be an optimum for a balance between repulsive and attractive forces between protein molecules. Shearing caused, that all dispersions had on the beginning a similar phase angle and storage modulus value (Fig. 3 and 4). Pre-shearing of whey protein polymers/aggregates (pH 6.5 and 7.0) resulted in a gel

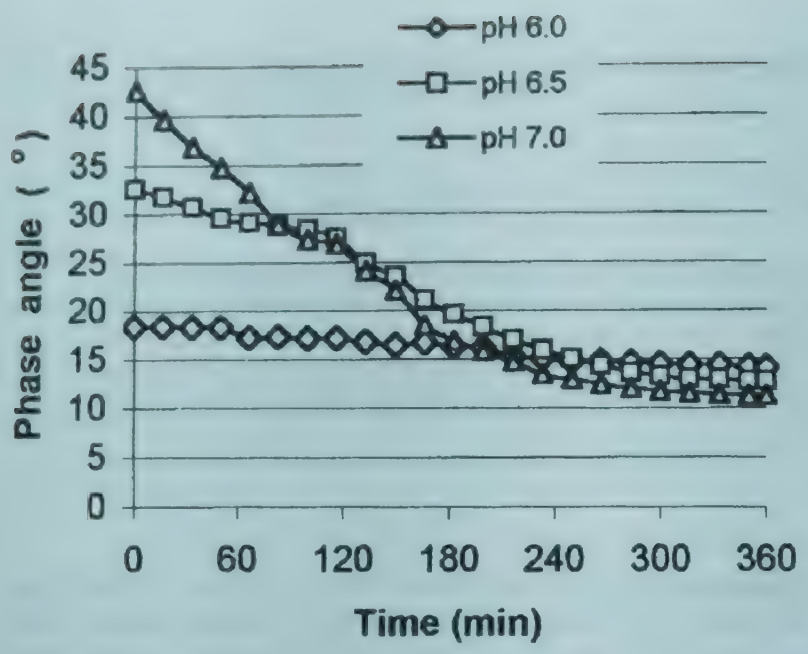

Fig 1. Phase angle changes with time for 3% (w/w) protein WPI dispersions (pH 8.0) heated at 80^0 for 50 min and adjusted to pH 6; 6.5 and 7 and heated for 1 h at 80^{0}C.

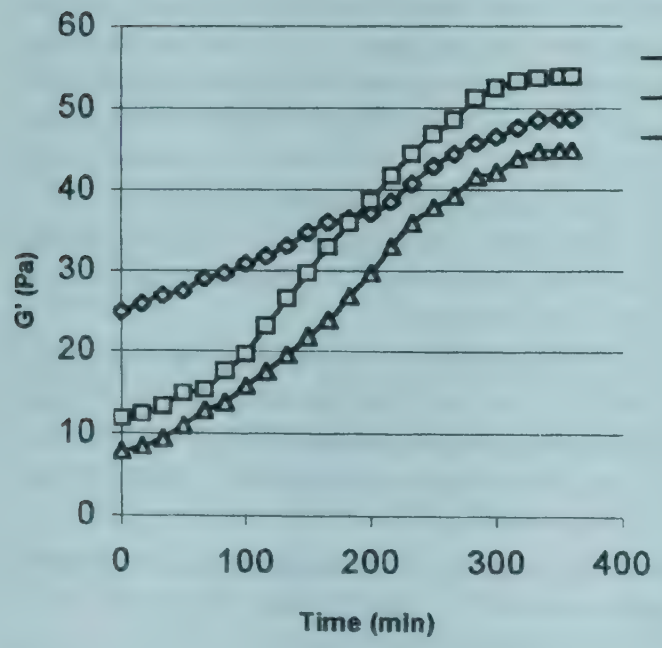

Fig 2. Storage modulus changes with time for 3% (w/w) protein WPI dispersions (pH 8.0) heated at 80^{0}C for 50 min and adjusted to pH 6; 6.5 and 7, and heated for 1 h at 80^{0}C.

with a lower phase angle and a higher storage modulus than for gels obtained from not-sheared dispersions. Nae and Reichert (1992) investigated lightly crosslinked carboxy copolymers in aqueous solutions and found that the recovery of both viscosity and storage modulus after shearing was very fast. It was related to the very fast rearrangements of the microgel structure as a function of time. Shear has effects on composition fluctuations and phase separation. There is an asymmetric stress division between the components of a mixture due to their size difference. The interaction network of particles can store elastic energy and while a fluid component can't (Tanaka 1999). The structure of a non-sheared gel is inhomogeneous and gel particles with the ability to store elastic energy are separated by the fluid phase, which results in higher

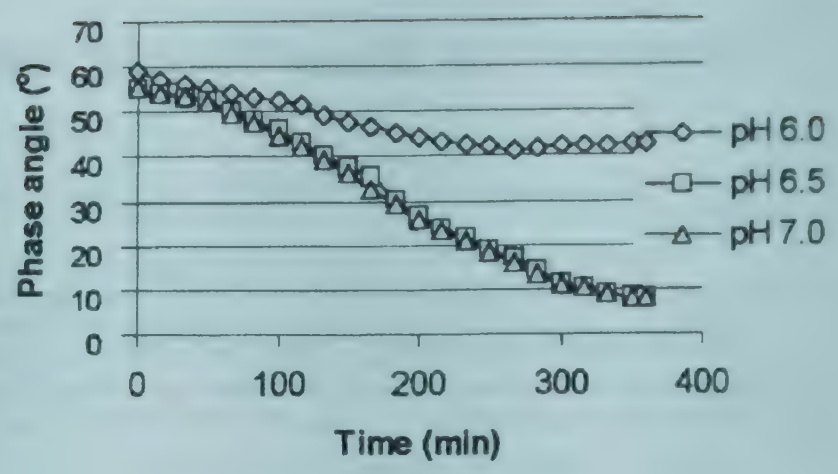

Fig 3. Phase angle changes with time for 3% (w/w) protein WPI dispersions (pH 8.0) heated at 80^{0}C for 50 min and adjusted to pH 6; 6.5 and 7, heated for 1 h at 80^{0}C and sheared for 5 min at 20,000 rpm.

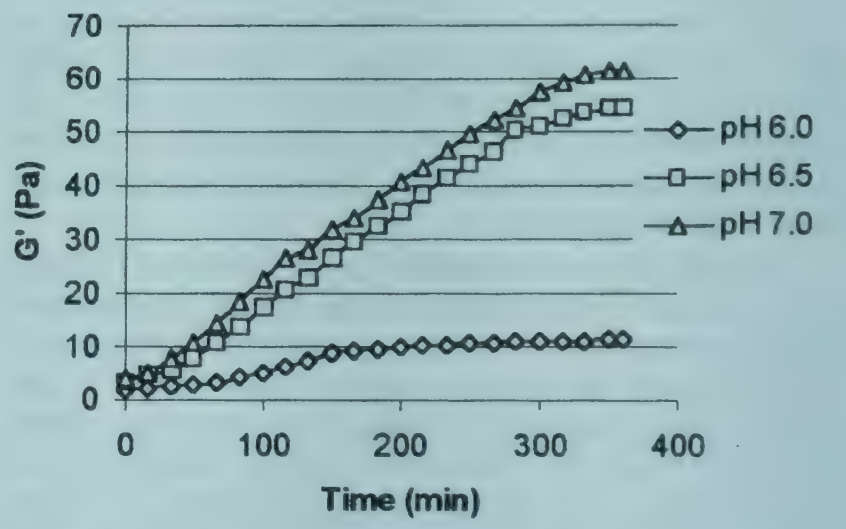

Fig 4. Storage modulus changes with time for 3% (w/w) protein WPI dispersions (pH 8.0) heated at 80^{0}C for 50 min and adjusted to pH 6; 6.5 and 7, heated for 1 h at 80^{0}C and sheared for 5 min at 20,000 rpm.

phase angle values. Shear causes redistribution of active sides engaged in gelation through the whole volume. The resulting gel has a more homogenous matrix, while still containing at least the same number of active sides. Such gels have a better capability to store energy under deformation, so is more elastic and is characterized by a higher elastic modulus. Muzny et al (1999) found that shear stresses induce a structural densifcation in gelling colloidal silica systems. A higher G; compared to that for the unsheared gel, was found for gels subjected to shear for short periods of time in the vicinity of the gel point (Walkenstrom et al. 1998 a). The authors suggest that shearing at pH close to the gel point affected the kinetics of the gel formation. Continuous shearing under stresses below 0.09 Pa, during heating from 20 to 60^{0}C and above, also resulted in gels with an increased G'. Continuous shear under stresses above 0.9 Pa resulted in gels with a decreased G'. This value was for non preheated samples. For preheated samples there was always a decrease in the G' independently of shear stress (Walkenstrom et al. 1998 a). Heating with continuous shearing probably caused

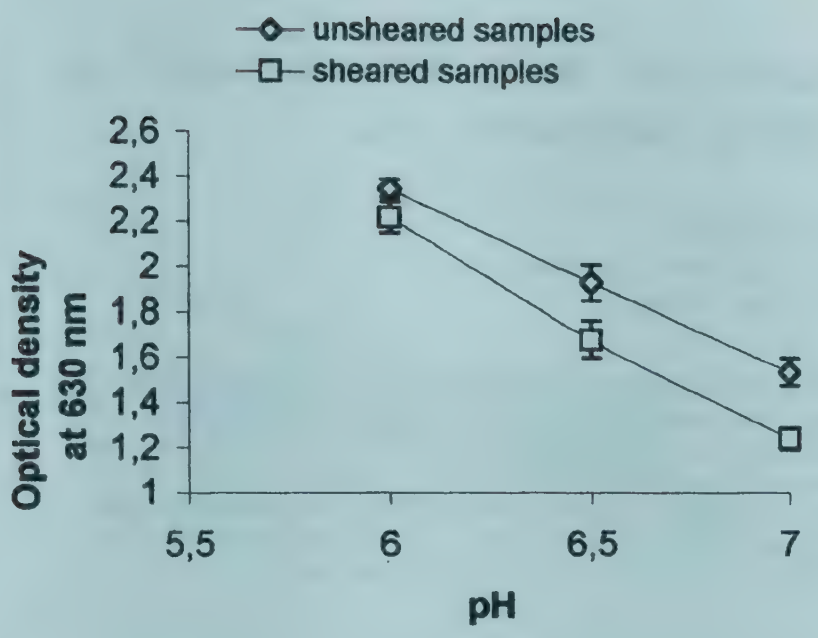

Fig. 5 Changes in optical density of the gel.

less aggregation in comparison to unsheared gels and obtained gels were better balanced in terms of interactions within and between aggregates, which resulted in higher G' value. Ker and Toledo (1992) reported that sheared suspensions gelled more rapidly than unsheared ones and produced stronger gels. SEM micrographs showed the development of a fine fibrous-like structure in the sheared suspensions, indicating numerous sites of protein-protein interactions.

The balance between repulsive and attractive forces between protein molecules is pH dependent. Gels obtained at a pH closer to neutral have well balanced repulsive and attractive forces between molecules. Gels obtained from pre-sheared dispersions at pH, 6,5 and 7.0 characterized by higher G' value (Fig. 4). Pre-shearing of the dispersions obtained at pH 6.0 resulted in a weaker gel. Walkenstrom et al (1998 b) found that shearing of particulate whey protein gels (pH 5.4) caused a decrease in G' and stress at fracture, indicating aggregate break-up during the steady shear.

The optical density showed a linear decrease with increasing pH in the range of 6.0 to 7.0 (Fig. 5). This is consistent with an increase in pH causing a reduction in aggregate size (Hoffmann 1997), and therefore, less light scattering and turbidity. Un-sheared gels had a higher optical density than pre-sheared gels, which suggests the formation of a gel composed of larger aggregates, which for pre-sheared gels were partially destroyed at the homogenization process.

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RESEARCH NOTE

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Rheological Changes Due to Substitution of Total Milk Proteins by Whey Proteins in Dairy Desserts

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Dairy desserts with starch, k-carrageenan and total milk proteins were prepared and their rheological behaviour was compared with desserts obtained by substitution of milk protein by whey proteins. As whey protein desserts were held at 90^{0}C for 5 min, an increase in apparent viscosity was observed. Increase in the concentration of starch resulted in higher apparent viscosity. In comparison to whey proteins, total milk proteins produced desserts with lower apparent viscosity. Holding at 90^{0}C did not result in increase of apparent viscosity and there was smaller increase in apparent viscosity as the samples were cooled. Whey protein desserts had lower syneresis. All desserts had pseudoplastic and thixotropic flow behaviour. A mathematical model was applied to correlate effect of shear rate and time on apparent viscosity.

Keywords : Dessert, Rheology, Syneresis, Viscosity, Whey protein

Whey proteins are often used as functional ingredients in many foods. The most important proteins in whey protein isolates are : β-lactoglobulin, α-lactalbumin, bovine serum albumin and immunoglobulins. One of the most important functional properties of whey protein isolates, as a food component is their ability to form gels. Dispersions of heated whey proteins can form gels when salt is added before or after heating (Barbut and Foegeding 1993; Mleko and Achremowicz 1996; Mleko 2000 b). Utilization of whey protein concentrates and isolates by the food industry is still limited due to lack in our knowledge about the roles that compositional and physicochemical factors play in gelation.

Gelation optimizes applicability, stability and textural properties of end products. Production of milk desserts involves a step in which texturizing agents are added. Polysaccharides are widely used in dairy desserts as stabilizing, thickening and gelling agents. Different gums, starch, modified starch, gelatin and pectin are used to obtain desired textural properties of the final product. Some polysaccharides influence texture by modifying the rheological properties of the continuous aqueous phase, while others such as κ-and ι-carrageenans interact directly with the milk proteins. In the presence of milk proteins, gelation occurs at low κ-carrageenan concentration (Snoeren 1976). It appears, that κ-carrageenan interact specifically with κ-casein to form a complex which aggregates into a three-dimensional matrix. Mleko et al. (1997) found that whey protein isolate at a concentration of 3% did not form a self-supporting gel, but significantly enhanced the shear stress values of 0,5% κ-carrageenan, particularly at pH 6 to 7. The synergistic effect was greater for unheated milk than for unheated whey proteins, but was smaller for heated milk than heated whey proteins. Starch added to dairy desserts improved texture. Friedman (1995) found that addition of starch to egg white protein, increased hardness of gels obtained after heating. According to Hamann (1988) starch acts as a non-active filler, which causes higher degree of packing

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The objective of this investigation was to obtain dairy desserts with starch, κ-carrageenan and milk protein and to compare with desserts obtained by substitution of total milk protein by whey protein.

Desserts preparation and rheological measurements : Whey protein isolate (WPI) was obtained from DAVISCO Food Ingredients International (Le Sueur, MN, USA), skim milk powder was obtained from a grocery shop. The protein content was determined by macro Kjeldahl method (AOAC 1984). k-carrageenan was purchased from Sigma Chemical Co. Starch was obtained from National Starch and Chemical GmbH. Desserts were prepared by mixing 3% protein (milk or whey), 0.3% k-carrageenan and 1,2 or 3% starch. Dispersions were poured into Brookfield rotational viscometer DV-II+ and heated from 60 to 90^{0}C, held for 10 min at 90^{0}C and then cooled to 20^{0}C. Apparent viscosity was measured at the shear rate 18.6 s^{-1}. For a dessert with 3% protein, 0.3% κ-carrageenan and 2% starch the apparent viscosity was measured at 20, 30, 40, 50, 60, 70, 80 and 90^{0}C at the shear rate range (0.3 to 95 s^{-1}) for 5 min at every shear rate, and multi-linear regression was used to find coefficients of equations :

$$\text{app. visc.} = (\text{shear rate})^a\ (\text{time})^b$$

Measurement of syneresis : Dessert samples were centrifuged at 1000 rpm for 10 min. using MPW-365 centrifuge (Mechanika Precyzyjna, Warsaw, Poland) and syneresis was calculated as percent of lost water.

Fig 1 shows influence of temperature on apparent viscosity of desserts prepared by mixing 3% whey protein, 0.3% κ-carrageenana and 1, 2 or 3% starch. As the samples were held at 90^{0}C for 5 min, an increase in apparent viscosity was observed. This was probably caused by aggregation of whey proteins due to formation of disulfide bonds and hydrophobic interactions and interactions of whey proteins with κ-carrageenan and starch. Cooling down from 90 - 20^{0}C resulted in steady increase in apparent viscosity, with an abrupt increase below 28 - 25^{0}C. Higher concentration of starch resulted in higher apparent viscosity. Increase in apparent viscosity at lower temperature is a standard behaviour of all

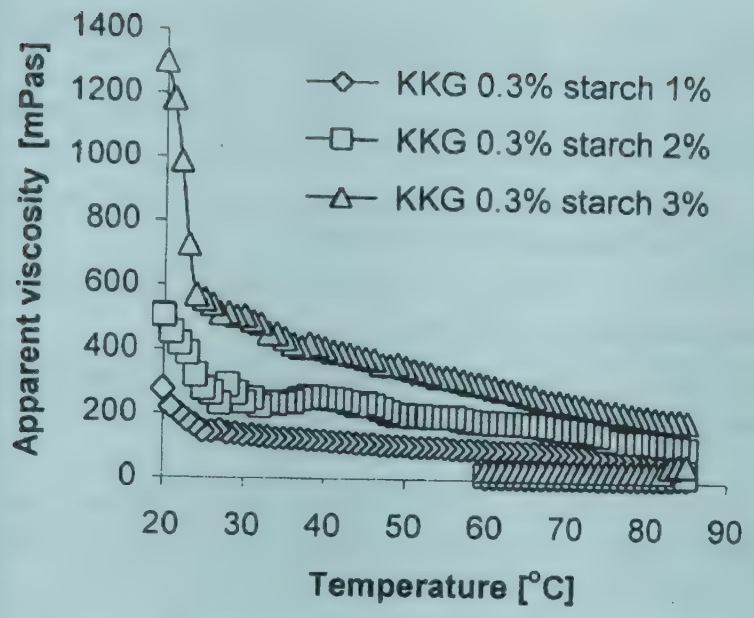

Fig. 1. Apparent viscosity of 3 % (w/w) whey protein dessert with 0.3% κ-carrageenan (KKG) and 1,2 and 3% starch heated from 60 to 90°C, held for 10 min at 90°C and then cooled from 90 to 20°C

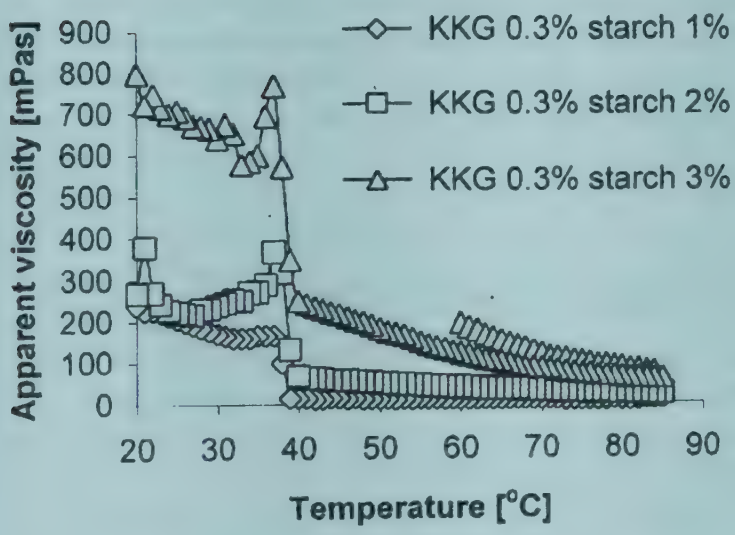

Fig. 2. Apparent viscosity of 3 % (w/w) total milk protein dessert with 0.3% κ-carrageenan (KKG) and 1,2 and 3% starch heated from 60 to 90°C, held for 10 min at 90°C and then cooled from 90 to 20°C

polysaccharide and protein gels (Mleko 1997). It is caused by lowering of kinetic energy of molecules and formation new bonds, especially hydrogen ones. An abrupt increase in viscosity at temperatures below 28-25°C might be as a result κ-carrageenan gelation. κ-carrageenan forms very strong gels, and its gelation temperature depends on environmental conditions. In comparison to whey proteins, total milk proteins produced desserts with lower apparent viscosity (Fig. 2). Holding at 90°C did not result in an increase in apparent viscosity and there was smaller increase in apparent viscosity as the samples were cooled. At about 40°C, an abrupt increase of apparent viscosity was noted, probably due to gelation of κ-carrageenan. Similar findings were noted for whey and total milk proteins desserts investigated by small strain dynamic rheology (Mleko 1997). Holding of whey proteins desserts at 90°C resulted in increase of storage modulus and decrease in phase angle. This low phase angle

TABLE 1. EFFECT OF DESSERT COMPOSITION ON SYNERESIS

	κ-carrageenan (%)	Starch (%)	Syneresis (%)
Whey protein isolate	0.3	1	2.5
	0.3	2	0.1
	0.3	3	0
Total milk	0.3	1	4
	0.3	2	0.4
	0.3	3	0.3

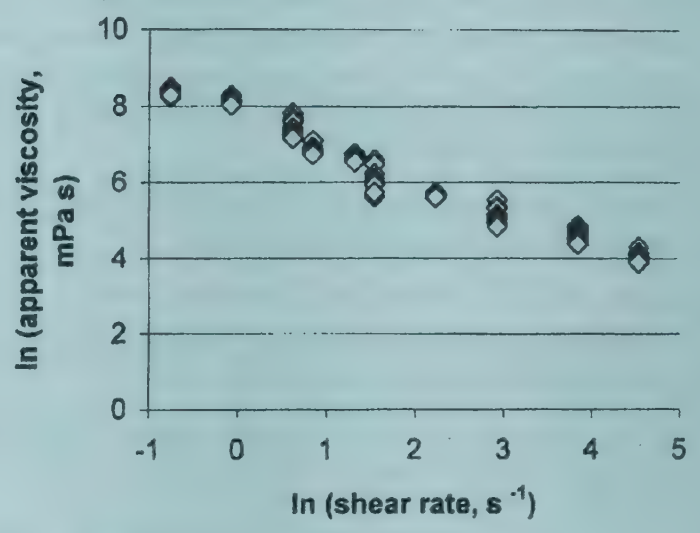

Fig. 3. Apparent viscosity vs shear rate behavior of 3 % (w/w) whey protein dessert with 0.3% κ-carrageenan (KKG) and 2% starch. (Samples were held at every shear rate for 5 min. A new sample was used for every shear rate).

TABLE 2. EFFECT OF TEMPERATURE ON THE COEFFICIENTS OF THE MATHEMATICAL MODEL

Temp.°C	a	b	R^2
20	-0.57	-0.41	0.96
30	-0.55	-0.72	0.94
40	-0.78	-0.09	0.98
50	-0.60	-0.55	0.98
60	-0.57	-0.10	0.96
70	-0.56	-0.43	0.98
80	-0.76	-0.20	0.97
90	-0.59	-0.32	0.97

Apparent viscosity = (shear rate)^a (time)^b

value indicated a high elastic component of the WPI desserts. It seems, that a critical condition for dessert production is its holding at 90°C. Interactions between whey protein, κ-carrageenan, starch and polymerization/aggregation of whey proteins, result in a gel formation. Lowering of temperature results in more dynamic increase of viscosity in comparison

to total milk proteins (Mleko et al. 1997). Different behaviour of whey protein desserts in comparison to total milk desserts can partially be caused by different protein content in whey protein isolate (91.7 %) and skim milk powder (36.7 %). Higher concentration of lactose in skim milk powder could hinder aggregation of milk proteins. Whey protein desserts had lower syneresis (Table 1), which is an important feature from the point of view of acceptability and shelf life of the product.

All desserts had pseudoplastic and thixotropic flow behaviour (Fig. 3). A mathematical model was applied to correlate the effect of shear rate and time on apparent viscosity (Table 2). At all temperatures studied, negative values of equation coefficients were noted, which supports observed pseudoplastic and thixotropic behaviour. Pseudoplastic and thixotropic behaviour of heated whey protein dispersions was also observed by Mleko and Foegeding (2000). Shear forces result in disruption of gel structure, but previous results suggest that most junctions between molecules in weak whey protein gels are reversible (Mleko 2000 a).

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RESEARCH NOTE

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Prevalence of *Listeria* spp. in Seafood Handlers

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The seafood handlers mainly peelers and processors in the seafood processing plants located at Mangalore, India were screened for the presence of *Listeria* spp. in their hands. About 4% of the seafood handlers were found to carry *L. monocytogenes*. Floors, drains, tables, containers and equipments of the seafood processing plants were also examined for the incidence of *Listeria* spp. and found that 5.1% of the samples were positive for *L. monocytogenes*. Results suggest that the sanitary and hygienic practices followed in these processing plants are not proper and adequate and it should be viewed seriously as there is a possibility that the final processed frozen seafood products may get contaminated with *L. monocytogenes* from these handlers and contact surfaces.

Keywords : *Listeria monocytogenes*, Prevalence, Seafoods, Handlers, Processing plants

Seafoods are gaining popularity and the demand for ready-to-eat foods are ever increasing and the problems associated with *Listeria monocytogenes* in seafoods are also increasing. Seafoods have been implicated in several sporadic cases of listeriosis (Facinelli et al 1989; Frediksen 1991; Baker et al. 1993) and in two outbreaks (Lennon et al. 1984; Riedo et al. 1990). The industry costs for contamination of a product with *L. monocytogenes* can be extremely prohibitive. The USFDA requires that *L. monocytogenes* is absent in ready-to-eat foods including seafoods (Fuchs and Reilly 1992). The isolation of *L. monocytogenes* from seafood products that have supposedly undergone listericidal process suggests that the products have been contaminated after processing due to unsanitary and unhygienic conditions in the processing plants. Kerr et al (1993) reported the prevalence of carriage of *L. monocytogenes* on the hands of fishmongers who handle raw seafoods. It has been reported that the *L. monocytogenes* was present in the surface of a seafood processing plant (Knight et al. 1998). Fuchs (1991) isolated *L. innocua* from various locations of the shrimp processing plants located in a Southeast Asian country. The species of *Listeria* is more frequently isolated from the environment than *L. monocytogenes*, but it is possible that the pathogen may also be present. Against this background, the prevalence of *Listeria* spp. particularly *L. monocytogenes* in seafood handlers and in various surfaces of seafood processing plants was studied.

Swabs taken from the hands of seafood processing plant workers and various surfaces of seafood processing plants located at Mangalore, India were immediately placed into enrichment broths. The protocol followed for the isolation of *Listeria* spp. from swab samples was adopted from USDA (McClain and Lee 1988) USFDA (Lovett et al. 1987) and NGFIS (van Netten et al. 1989) methods. The swabs were placed into 10 ml of USDA Listeria enrichment broth (UVM 1) and incubated at room temperature (28 ± 2^0C) for 24 h for primary enrichment. Then, 0.1 ml of the primary enriched sample was transferred to 10 ml of Modified Fraser broth (MFB) and incubated at 37^0C for 18 to 24 h for secondary enrichment. The secondary enriched samples were streaked onto PALCAM (Polymyxin Aesculin Lithium Chloride Ceftazidime Acriflavine Mannitol) agar plates and incubated at 37^0C for 48 h. Typical colonies were picked up and purified onto trypticase soy agar with 0.6% yeast extract and then subjected to various biochemical tests for characterization and identification (McLauchlin 1987; Lovett 1988; Jones and Seeliger 1992).

Of the 50 seafood handlers (peelers and processors) working in two seafood processing plants, which mostly process frozen shrimps, squids and cuttlefishes for the export, examined for the carriage of *Listeria* spp. in their hands, two were found to carry *L. monocytogenes* (Table 1). Among various surface samples screened from the seafood processing plants for *Listeria* spp., only three samples were positive. Two samples from peeling/processing tables and one from plate freezer yielded only *L. monocytogenes*.

The results show that 4% of the seafood handlers examined were found to carry *Listeria* spp. in their hands. Only *L. monocytogenes* was isolated from these seafood processing workers who handle, peel, clean and process shrimps, squids and cuttlefishes for freezing for export purposes. Since the seafood handlers did not have any infection in their hands, the contamination was derived during the handling operation, possibly from raw shrimps. The results suggest that there is a possibility of cross contamination of the *Listeria*-free foods through the hands of these handlers. Kerr et al (1993) reported that 7 % of fishmongers who handle raw seafoods carried *L. monocytogenes*. Even hand washing was not successful in eliminating listeriac, if they were present in larger numbers (>20 cfu). The prevalence of *L. monocytogenes* on the hands of seafood processing plant workers who handle shrimps, squids, cuttlefishes meant for export in frozen condition indicates the improper personnel hygienic practices followed in these processing plants and should be viewed seriously as there is every possibility that the final processed frozen seafood products may get contaminated with *L. monocytogenes* from

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TABLE 1. INCIDENCE OF *LISTERIA* SPP. IN ENVIRONMENTAL SWAB SAMPLES OF SEAFOOD PROCESSING PLANTS

Type of samples	No. of samples tested	Number of samples positive for *Listeria* spp.	*Listeria monocytogenes*	*Listeria welshimeri*
Floors	11	—	—	—
Drains	2	—	—	—
Tables	12	2	2	—
Utensils/ containers	27	—	—	—
Equipments/ machineries	7	1	1	1
Workers' hands	50	2	2	—
Total	109	5	5	1

these workers.

Results also show that 5.1% of the surface samples collected from seafood processing plants were positive for *L. monocytogenes*. However, Mylvahanan (1991) and Fuchs (1991) isolated *L. innocua* from peeled shed of seafood processing plants and from containers, trays, tables, weighing equipment, floors, drains and workers' boots of seafood processing plants. This species of *Listeria* is more frequently isolated from the environmental samples than *L. monocytogenes*. but it is possible that the pathogen may also be present. The incidence of *L. monocytogenes* in plate freezer is a significant risk factor, as it can contaminate the seafoods that are frozen in the plate freezer. Though the initial contamination of plate freezer with *L. monocytogenes* may be from contaminated raw seafoods that are being frozen, *L. monocytogenes* can attach to the surface of plate freezer firmly which will be resistant to cleaning with sanitizers (Ronner and Wong 1993). The occurrence of *L. monocytogenes* on processing tables also suggests that raw seafoods contain *L. monocytogenes* and contaminate these surfaces while handling and further processing. *L. monocytogens* can form biofilms on these contaminated surfaces on long exposure to raw seafoods which carry this pathogen (Fuchs and Reilly 1992). Because of its nature to form biolfilms (Costerton et al. 1987), *L. monocytogenes* could be found from these surfaces, even after regular cleaning and sanitizing the surfaces of seafood processing plants. Such contaminated surfaces may cross-contaminate the seafoods, that were initially free from this pathogen, which will lead to health hazard on consumption of such contaminated seafoods. The incidence of *L. monocytogenes* on plate freezer, processing table and seafood handlers' may have serious impact on the cleaning and sanitizing the surfaces of seafood processing plants and also in the processing and production of frozen seafoods for export.

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RESEARCH NOTE

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Lipid Composition of Bivalve *Pelecyora trigona*

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Lipid composition of the bivalve *Pelecyora trigona* indicated that the total lipid (0.9 ± 0.1%) contain 50 ± 8.4% phospholipid and 13.2 ± 3.5% cholesterol as major constituents. The ratio of phospholipid to cholesterol of *P. trigona* is 3.76. The fatty acid composition of total lipids revealed that saturated fatty acids constitute 56.15 ± 0.35% followed by polyunsaturated fatty acids (37.6 ± 1.83%) and monounsaturated fatty acids (10.25 ± 2.19%). Palmitic acid ($C_{16:0}$) was the major (43.5 ± 0.2%) saturated fatty acid. Among the polyunsaturated fatty acids, eicosapentaenoic acid $C_{20:5}$ (15.6 ± 0.00%) was present in significant amount.

Keywords : Bivalve *Pelecyora trigona*, Lipid composition, Phospholipid, Cholesterol, Fatty acid composition.

The current interest in the role of dietary polyunsaturated fatty acid particularly that of eicosapentaenoic acid (20 : 5_{n-3}) in amelioration of certain cardiovascular diseases (Dyerberg et al 1975, 1978, Dyerberg and Bang 1979) focuses attention to marine fishery products which are excellent sources of polyunsaturates. Bivalves comprising mussels, scallops, oysters and clams are valued sea foods and are of greatest commercial importance (Joseph 1982). Lipid constitutes an important fraction of molluscan tissue (Voogt 1972). The basic type of lipids found in mollusca include sterol, phospholipids and fatty acids (Voogt 1983). *Pelecyora trigona* are found in abundance in Canning estuarine area of the Bay of Bengal. The present investigation deals with the fatty acid compositions of the total lipid of the bivalve *P. trigona* with a view to ascertain its dietary importance.

The bivalve *P. trigona* were collected form Canning estuarine area of the Bay of Bengal during winter time (December) and were studied for lipid and fatty acids. The lipids were extracted from each bivalve according to the process of Bligh and Dyer (1959). As many as eleven individual bivalves (*P. trigona*) were extracted. The isolated lipids from each bivalve were dissolved in chloroform and stored at 0^0C (Kates 1972) for analysis immediately after extraction. The entire analyses were completed within one week. Phospholipid and cholesterol in bivalve lipid were estimated according to the standard method of Chen et al 1956) and Zlatkis et al (1953), respectively. Fatty acid composition of lipid samples were analysed by gas liquid chromatography (model HP-5890 A Hewlett Packard) equipped with flame ionization detector.

Methyl esters of the total lipid samples were prepared following the method of Litchfield (1972). Each methyl ester derived from each bivalve lipid was isolated in pure state by using preparative silica gel TLC technique using n-hexane/ diethyl ether / acetic acid (80 : 20 : 1, v/v/.v) as eluting solvent. Visualisation was effected by exposing the plates to U.V. lamp. Fatty acid ester peaks were identified by the traditional practice of running the methyl ester of standard saturated and unsaturated fatty acids of various chain length and degree of unsaturation. Also the use of cod-liver oil fatty acids methyl ester was made as a secondary standard (Ackman and Burgher 1965) as recommended by Ackman.

The total lipid content and the proportions of the structural lipids like phospholipid and cholesterol in *P. trigona* are presented in Table 1. The phospholipid content of total lipid was high (50.01% ± 8.4) whereas cholesterol content was far low (13.27% ± 3.5) of total lipid. The total lipid of bivalves species such as *Macoma* sp. (0.5%) (Misra et al. 1985) and *Anadara granosa* (0.65%) collected from near about zones has shown lower total lipid content. The commonly found distributions of the major fatty acid moeties ($C_{16:0}$, $C_{18:0}$, $C_{20:5}$) in marine oils have been observed also in *P. trigona*. The fatty acid compositions of molluscs have been amply studied by several groups (Voogt 1983, Tibaldi 1966, Ackman and Hooper 1973, Johnes et al. 1980, Gardner and Riley 1972). Also total saturated fatty acids are high in *P. trigona*. Saturated fatty acids have been indicated as primary source of energy in bivalves and gastropods (Polok et al. 1987). The principal fatty acids in these organisms have been recognized to be saturated acids, palmitic (16 : 0) and stearic (18 : 0) (Table 2) (Carballeira and Anstaci 1992) which is also observed in the present study with *P. trigona* where $C_{16:0}$ was 43.5% ± 0.2 of lipid and $C_{18:0}$ was 4.85% ± 0.2. $C_{16:0}$ aid in synthesis of cellular neutral lipids (Kanazawa et al. 1979). Monounsaturated fatty acid constitute the least fraction of fatty acids. $C_{20:5}$ is unusually high in this bivalve and its presence in such high amount (15.6% ± 0.0) nullifies the effect of the presence of $C_{16:0}$ in such great amount. Voogt (1972) stated that bivalves show a typical marine distribution of their fatty acids 20:5 and 22:6 being the most important unsaturated acids. $C_{22:5}$ is also

TABLE 1. TOTAL LIPID AND STRUCTURAL LIPID COMPOSITION OF *PELECYORA TRIGONA*

Total lipid (%w/w of tissue)	0.9 ± 0.1
Total cholesterol (% of total lipid)	13.2 ± 3.5
Phospholipid (% of total lipid)	50.0 ± 8.4

n (no. of bivalves) = 11

Mean ± standard deviation

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TABLE 2. FATTY ACID COMPOSITION OF THE WHOLE BODY TOTAL LIPID OF THE BIVALVE *PELECYORA TRIGONA*

Fatty acid	% w/w
	Saturated
14 : 0	5.25 ± 0.49
16 : 0	43.5 ± 0.28
18 : 0	4.85 ± 0.21
22 : 0	2.55 ± 0.07
Total	56.15 ± 0.35
	Monoenoics
14 : 1	1.00 ± 0.28
16 : 1	2.09 ± 2.12
18 : 1	6.30 ± 0.21
Total	10.25 ± 2.19
	Polyenoics
18 : 3	0.8 ± 0.00
18 : 4	5.8 ± 0.84
20 : 4	2.2 ± 0.00
20 : 5	15.6 ± 0.00
22 : 4	0.9 ± 0.14
22 : 5(n-6)	4.55 ± 0.49
Others	3.75 ± 1.34
Total	33.6 ± 1.83

n (no. of bivalves) = 11
Mean ± standard deviation

present in considerable amount in this bivalve. The occurrence of $C_{20:4}$ in low (2.2% ± 0.0) amount in *P. trigona* lipid could be due to salinity factor.

Fatty acid composition of marine bivalves can be affected by salinity. Low salinity conditions result in the increased content of arachidonic acid ($C_{20:4}$) in marine bivalves. Bivalves inhabiting condition of higher salinity also shows elevated content of $C_{20:5}$ acid (Wenne and Polak 1992). So presence of low $C_{20:4}$ (2.2% ± 0.0) and high $C_{20:5}$ (15.6% ± 0.0) shows that the bivalve is highly marine in nature inhabiting in higher salinity condition. *P. trigona* consumption is highly nutritious for human health as well as cheap for its abundant availability in estuarine area of Gangetic West Bengal.

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RESEARCH NOTE

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Effect of Dietary β-Carotene on the Animal Performance and Fatty Acid Composition of Adipose Tissue of Calf

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The fatty acid composition of adipose tissue was measured in 9 Black Pied calves to determine β-carotene effect. Calves allotted to three groups were fed the diets containing 2 levels of β-carotene, during the fattening period. All groups were fed *ad libitum* for six months and slaughtered. Adipose tissue samples were taken from subcutaneous, kidney, pelvic and abdomen and fatty acid composition was determined using gas chromatograph. When β-carotene level was increased in the feed, the fat of calves became softer and their colour became yellow. The palmitic, oleic percentages changed on feeding β-carotene and changes in fat location were significantly different ($P < 0.01$). Feeding β-carotene and fat location affected the total saturated and unsaturated fatty acid percentage significantly ($P < 0.01$). In abdomen and pelvic fat, there was positive correlation between feeding β-carotene and total unsaturated fatty acids and the ratio of unsaturated to saturated fatty acids whereas the correlation between total saturated fatty acids and β-carotene were negative. Addition of β-carotene in feed has increased oleic acid percentages in all fat samples.

Keywords : β-carotene, Adipose tissue, Fatty acids, Calf.

Since it has been established that reducing the level of fats in human diet could play a preventive role against some diseases especially heart disease, levels of fats and their composition in beef have gained much attention. The modern, genetic approaches try to change the fatty acid composition of the beef livestock to reduce palmitate levels and increase oleats due to better health impacts of this composition on human beings (Huerta-Leidenz et al. 1993).

There have been several studies showing that the composition of fatty acids in beef products is closely linked with the way the livestock's are fed (Hidiroglou et al. 1987, Monahan et al. 1992, Mandell et al. 1997, 1998).

It has been shown that mono-unsaturated fatty acid levels have increased with the increasing levels of β-carotene in cattle, grazed on pasture (Zhou et al. 1993). In the cattle, grazed on pasture, β-carotene concentration in subcutaneous fat, liver and rumen has been found 0.81; 7.01 and 8.8 mg/kg respectively (Yang et al. 1992). β-carotene has the highest provitamin A activity of the carotenoids. However, the efficiency of conversion of carotene to vitamin A differs with species. For example 1 mg of β-carotene is equivalent to 251-500 IU of vitamin A in pig. The conversion efficiencies are three to six times lower for poultry and rats (Chew 1993). As well as being an effective provitamin, β-carotene also shows important degree of antioxidant characteristics in low oxygen pressure (Burton and Inglond 1984, Yamauchi et al. 1993). It has been shown that β-carotene, which is wide spread in nature, has a preventive effect against cancer due to its ability to react with free radicals (Block and Langseth 1994, Singh et al. 1997). β-Carotene absorbed in small intestines is transported to liver and adipose tissues where it changes colour of the fat to yellow. In many countries the colour of fat tissue is an important factor for beef grading. As a result, high levels of β-carotene concentration in fats reduce the value of the meat products causing economic losses (Yang et al. 1992). In spite of this negative aspect, the fact that β-carotene increases especially unsaturated fatty acid levels has drawn a significant attention.

For this reason, calves raised under controlled farm conditions have been fed two levels of β-carotene and a relationship between fatty acid composition of adipose fat and β-carotene has been investigated. Calves have not been grazed on pasture and they have been fed with barley, cotton seed meal and supplemental β-carotene.

Animals and their management : A total of 9 Black Pied calves, aged 6-12 months were used in the trial. They were fed at Ankara University Research application farm. Their initial weight was approximately 218 kg. Calves were allotted at random in equal numbers to three groups. One group was separated as a control group and fed with 75% barley, 10% cottonseed meal, 10 % wheat bran, 2.5% calcium carbonate, 1% mineral-vitamin pre- mixture, 0.5% salt. The composition of the mineral mixture was 10000 mg manganese, 10000 mg iron, 10000 mg zinc, 5000 mg copper, 100 mg iodine, 100 mg selenium, 369.6 mg calcium carbonate / kg mixture. The composition of the vitamin mixture was 15000000 international unit (IU) vitamin A, 3000000 IU vitamin D_3, 30 g vitamin E, 5 g vitamin B_1, 10 g niacin per kg of mixture. Second group was fed with control group feed plus 6.0 mg of β-carotene / kg feed. Third group was fed with control group feed plus 12.0 mg of β-carotene / kg feed. Additional, each animal in all groups was fed with 1.5 kg hay per day. All groups were fed *ad libitum* for six months.

Before the slaughter, animals were weighed at monthly intervals and final live weight, feed consumption, daily gain and feed/gain ratio of animal were determined.

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The animals were slaughtered in the experimental slaughter house facilities. Samples of fat were taken from subcutaneous, kidney, pelvic and abdomen. A subcutaneous fat sample was taken from 12-13th ribs. All samples were stored at -20^{0}C until required for analysis.

Fatty acid analysis : Samples of fats were esterified with boron tri fluoride/ methanol as described in AOAC (1990). Esterified samples were extracted with hexane and then analyzed using a flame ionization gas chromatograph (Shimadzu GC-15) equipped with a 30 meter Supercowax capillary column. The column was run isothermally at temperature 230^{0}C. Chromatograms were recorded with a computing integrator (Shimadzu C-R4A).

Statical analysis : The individual fatty acid data was grouped as total saturated and total unsaturated, after mean percentage of individual fatty acids obtained from three calves in each treatment was determined. The ratio of unsaturated to saturated fatty acids was calculated. Variance analysis was applied to these data using the statistica packed program (StatSoft. Inc., 1995). Feeding method and fat location were used a main effect in statistical evaluation. LSD (least significant difference) test was used to compare means and correlation was made between feeding method and total fatty acids in different fat location.

Animal and carcass data : Performance data and carcass characteristics of calves are presented in Table 1. Average daily gain for control; second and third group was 1.23, 1.19 and 1.55 kg/head/day, respectively. This difference was not significant (P>0.05).

After slaughter, when the carcass fats were analyzed for their colour and consistency characteristics, it was found that in line with increased levels of β-carotene the fat became softer and their colour became yellow. These relations were significant (P<0.01) (Table 1).

Fatty acids : The variation of fatty acid percentages based on feeding method and fat locations has been analyzed using variance analysis. Feeding method has made significant effect on myristic, palmitic, stearic, arachidic and oleic acid (C14 : 0, C16 : 0, C18 : 0, C18 : 1, C20 : 0) percentages. Different fat locations have affected on oleic, palmitic and stearic acid percentages. Lionoleic and linolenic acid (C18 : 1, C18 : 2) percentages have not been affected from different fat locations or methods at all. None of the fatty acids has been observed to have any interaction with either fat locations or feeding method (Table 2).

When the total saturated and unsaturated fatty acids were analyzed, based on the feeding method and fat location, it was found that feeding method and fat location affected the total saturated and unsaturated fatty acid percentage significantly (Table 3).

In LSD tests for each fatty acid analyzed, fatty acid percentage were separated into two groups, based on feeding methods. When the addition of β-carotene, average of myristic, palmitic, stearic and arachidic acid percentages have decreased. Percentages of oleic acid however, increased with the addition of β-carotene (Table 4).

In LSD test based on fat location, means of fatty acid percentage were separated two and three groups. In subcutaneous fat, mean of palmitic and stearic acid percentages were found to be the lowest, whereas the oleic acid percentages were the highest. In pelvic fat, means of palmitic and stearic acid percentage were found to be the highest, whereas the oleic acid average levels were the lowest (Table 5).

When the total saturated and unsaturated fatty acid

TABLE 1. INFLUENCE OF SUPPLEMENTAL β-CAROTENE ON ANIMAL PERFORMANCE AND CARCASS CHARACTERISTICS OF CALVES

Item	Groups			F
	Control	6 mg β-carotene per kg feed	12 mg β-carotene per kg feed	
Initial weight, kg	230.8	230.9	218.5	
Daily gain, kg	1.23	1.19	1.55	NS
Feed/animal/day (as fed), kg	6.85	6.74	6.91	
Feed/grain ratio (as fed), kg	6.2	7.5	6.5	
Live weight, kg	438	459	479	
Hot carcass weight, kg	233	253	272	
Dressing percentage	53.0	54.6	57.9	6.83*
Colour of fat[a]	1.3	2.7	3.6	12.33**
Consistency of fat[b]	2	3.3	3.7	10.50**

[a]Fat color : 1 = white, 2 = cream, 3 = slightly yellow, 4 = yellow.
[b]Consistency of fat : 1 = very hard, 2 = hard, 3 = slightly soft, 4 = soft.
NS = non-significant. * (P < 0.05); ** (P < 0.01)

TABLE 2 PERTINENT MEAN SQUARES FROM THE ANALYSIS OF VARIANCE FOR THE EFFECT OF FEEDING METHOD AND FAT LOCATION ON FATTY ACIDS

Source of variation	df	C14 : 0	C16 : 0	C18 : 0	C18 : 1	C18 : 2	C18 : 3	C20 : 0
Feeding method	2	2.75*	91.29**	319.23**	589.47**	2.46^{NS}	0.03^{NS}	0.29*
Fat location	3	0.47^{NS}	57.77**	204.37**	383.47**	0.97^{NS}	0.05^{NS}	0.11^{NS}
Feeding method X fat location	6	0.16^{NS}	19.49^{NS}	15.21^{NS}	30.14^{NS}	0.59^{NS}	0.03^{NS}	0.15^{NS}
Error	24							

NS (P ≥ 0.05) ; * (P < 0.05); ** (P < 0.01)

TABLE 3. PERTINENT MEAN SQUARES FROM THE ANALYSIS OF VARIANCE FOR THE EFFECT OF FEEDING METHOD AND FAT LOCATION ON TOTAL SATURATED AND UNSATURATED FATTY ACIDS

Source of variation	df	SFA	UFA	UFA/SFA
Feeding method	2	792.47**	803.34**	14.16**
Fat location	3	441.19**	388.83**	7.43**
Feeding method X Fat location	6	65.70^{NS}	44.63^{NS}	2.04^{NS}
Error	24			

NS ($P \geq 0,05$); * ($P < 0.05$); ** ($P < 0.01$)

TABLE 4. MEAN PERCENTAGE[1] OF FATTY ACIDS OF FAT SAMPLE IN DIFFERENT FEEDING METHODS

Feeding method	Means[(2)]						
	C14 : 0	C16 : 0	C18 : 0	C18 : 1	C18 : 2	C18 : 3	C20 : 0
Control	3.215a	48.290a	17.796a	55.912a	4.36	0.39	0.50a
Addition of 6 mg β-carotene/kg feed	3.879b	13.854b	9.006b	68.230b	5.16	0.46	0.23b
Addition of 12 mg β-carotene/kg feed	4.144b	13.233b	8.725b	68.321b	4.41	0.37	0.24b

(1) Percentage of total peak area of the fatty acids listed

(2) Mean values followed by the same letter for each variable is not significant at $P \geq 0.05$ level.

TABLE 5. MEAN PERCENTAGE[1] OF FATTY ACIDS OF FAT AT DIFFERENT FAT LOCATIONS

Fat location	Means[(2)]						
	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0
Abdomen	3.85	15.32ab	11.11b	61ab	4.94	0.44	0.23
Pelvic	3.25	18.52a	18.76a	55b	4.29	0.32	0.36
Kidney	3.96	13.78b	9.25b	67a	4.91	0.49	0.23
Subcutaneous	3.60	12.80b	8.23b	69a	4.43	0.36	0.46

(1) Percentage of total peak area of the fatty acids listed

(2) Mean values followed by the same letter for each variable is not significant at $P \geq 0.05$ level.

TABLE 6. CORRELATION BETWEEN FEEDING METHOD AND TOTAL FATTY ACIDS IN DIFFERENT FAT LOCATIONS

	Feeding Method			
	Abdomen	Kidney	Subcutaneous	Pelvic
Total saturated fatty acid %	-0.88*	-0.31	-0.45	-0.73*
Total unsaturated fatty acid %	0.84*	0.35	0.45	0.73*
Unsaturated/saturated ratio %	0.85*	0.37	0.42	0.70*

*($P < 0.05$)

percentage and the ratio of unsaturated to saturated fatty acid were analyzed for each fat locations separately, based on the feeding method, there were negative correlation between total saturated fatty acid and feeding method. The correlation between feeding method and both total unsaturated fatty acid and the ratio of unsaturated to saturated fatty acid was positive (Table 6). Correlation coefficients for abdomen and pelvic fat were significant whereas the correlation coefficient for subcutaneous and kidney fat were not significant. In all fat locations however, as the β-carotene levels were increased in feed, the total unsaturated fatty acid percentage and unsaturated / saturated ratio increased while decreasing total saturated fatty acid percentage. In other words, any increase in β-carotene level has increased monounsaturated fatty acid ratio while decreasing total amount of saturated fatty acid.

Among black pied calves under observation, the group that was fed with 12 mg β-carotene / kg feed have shown

increased levels of subcutaneous and deposit fat after slaughter, compared to the other groups. Also the colours of fat were more yellow. According to study performed by Zhou et al (1993) the colour of fats were found to be more yellow as the live stock were fed with increased concentrations of β-carotene. They have also shown that while there is a linear/ positive relationship with total cis mono-unsaturated fatty acids levels and β-carotene levels while saturated fatty acids and β-carotene levels are inversely related to each other. The same researchers have indicated that although there appears to be a tendency for linoleic acid levels to increase and myristic acid levels to decrease with the increasing levels of β-carotene, these relationships did not have any statistical significance. We have also reached similar conclusions in our studies as well. Huerta-Leidenz et al (1993) have analyzed the impacts of feeding methods and fat types on the composition of fatty acids on Brahman and Hereford cattle. They have indicated that the feeding method has a significant impact on C14 : 1, C16 : 0, C16 : 1, C18 : 1 with the exception of myristic acid. Moreover, fat thickness and fat thickness at the biopsy location was used as a covariate, fat thickness has significant effect on total saturated and unsaturated fatty acids and unsaturated/ saturated ratio while fat thickness at the biopsy location has not significant effect. Webb et al (1998) reported that monounsaturated fatty acids level in intermuscular fats varied with sample location.

In our study we have also found significant differences in C16 : 0, C18 : 0, C18 : 1 levels in different fat location.

In the studies on fatty acid concentrations, dietary fatty acid sources have protected unsaturated fatty acids from rumen biohydrogenation (Sing and Hawke 1990, Jenkins and Bateman 1996, Kelly et al. 1998). Studies on Angus and American Wagu steers have shown that stearoyl coenzyme A desaturase is responsible for conversion of saturated fatty acids to monounsaturated fatty acids. But enzyme activity is not different between 2 breeds (Cameron et al. 1994). Alan et al. whose results are reported by Zhou et al (1993), have found that β-carotene affects the activity of desaturate enzyme in the rat liver microsomes.

In the present studies, it has been found that β-carotene has increased oleic acid percentage significantly in black pied calf fat. The mechanism by which β-carotene influences on the desaturation of fatty acids has not been understood yet. This mechanism could be either by inhibiting hydrogenation or increasing the activity of the desaturase enzyme. It is certain however that β-carotene increases the concentration of oleic acid.

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Provitamin A Content of Selected South Indian Foods by High Performance Liquid Chromatography

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Ten green leafy vegetables, five other vegetables, five fruits in raw form and cooked greens were analysed for their total carotene contents by spectrophotometry and beta carotene/provitamin A content by HPLC. Selected foods were also evaluated for absorption of carotenes in humans. Dark green leafy vegetables were found to be the richest source of provitamin A. Drumstick leaves showed a maximum total - (41,98 mcg%) and beta-carotene - (28,16 mcg%) contents respectively. Among the vegetables and fruits studied, carrots and mangoes showed maximum carotene contents. Further, 81% of total carotenes in fenugreek leaves was beta carotene while in carrots, 87.8% was beta carotene and in mangoes 73.8% was beta carotene. Vitamin A activities of vegetables and fruits were found to be lower than leafy vegetables. The loss of beta carotene in cooked greens was in the range of 2.5 to 42.5% and beta carotene absorption was maximum in papaya (88.7%).

Keywords : Green leafy vegetables, Other vegetables, Fruits, Provitamin A, High performance liquid chromatography, Absorption.

Vitamin A deficiency is recognised as a serious public health problem leading to blindness. The World Health Organisation (WHO 1998) has estimated that 2.8 million pre-school children are at risk of blindness and the well-beings of 251 million people are affected due to vitamin A deficiency. Dietary interventions of including beta carotene rich foods have been recommended to be the best measure to improve the vitamin A nutrition of individuals. Though India has a wide range of inexpensive foods rich in carotenoids, the per caput availability of beta carotene in terms of retinol equivalents (400 mcg per day) is much below the recommended allowances of vitamin A for an individual (Vijayaraghavan and Nayak 1995). Open column chromatography commonly used for estimating different carotenoids is time consuming and unreliable.

Therefore, in this context High Performance Liquid Chromatography (HPLC) is considered to be the most reliable, efficient and reproducible method for carotenoid analysis (Zakaria et al. 1979; Beecher and Khachick 1984). As not much work has been carried on the beta carotene contents of carotene rich foods using HPLC in India, the present study was undertaken to analyse the provitamin A/carotene contents of selected green leafy vegetables, other vegetables and fruits consumed in Coimbatore, estimate the loss of carotenes in cooked forms and evaluate the absorption of carotenes from selected GLV, vegetable and fruit in adults.

Based on the results of the survey carried out by Chandrasekhar et al (1999) with regard to household consumption of carotene - rich foods in Coimbatore district, ten green leafy vegetables, five other vegetables and five fruits were identified and selected for the study. These foods were purchased from the local markets in Coimbatore. The foods selected for the study included *agathi,* drumstick leaves, fenugreek leaves, *manathakkali* leaves, *paruppu keerai,* amaranth (tender), *mulla keerai, araikeerai, ponnanganni* and spinach. The samples after purchase were brought to the laboratory, cleaned and edible portions were used for analysis within two hours.

Poriyal is the traditional meal form in which green leafy vegetables are consumed in South India. Hence the selected ten greens were cooked in standardised meal form namely greens *poriyal.* The method of preparation involved seasoning in groundnut oil, adding cut greens and cooking with required amount of water by absorption method and garnishing. Cooked greens were analysed for carotenes. All the analyses were carried out in triplicate.

For the absorption study, *mullakeerai,* drumstick leaves, carrot and papaya were chosen due to their popularity and maximum beta carotene retention. Ten adult women (between 20 and 24 years) in four groups (40) were selected based on their willingness, health status and co-operation. Balance study was conducted as per the procedure of Lala and Reddy (1970). During the repletion period of 10 days, the subjects belonging to group I, II, III and IV received 15 g of drumstick leaves *poriyal,* 20 g of *mullakeerai poriyal,* 51 g of carrot *poriyal* and 260 g of papaya daily, in addition to the basal diet (devoid of beta carotene). During the collection period, 24 h samples of faeces was collected (Rao and Rao 1970), weighed, blended and analysed for carotenes. One tenth of the food consumed during the basal and supplemental period was also analysed for carotenes. From the difference between the ingestion and excretion of total and beta carotene daily, the amount absorbed was computed for each individual.

Total carotenes : Total carotenes were estimated by the method of Zakaria et al (1979). Fresh samples were initially saponified using 12 % alcoholic potassium hydroxide and the carotene extract was prepared using petroleum ether (60 - 80^{0}C) as the solvent. The total volume of the extract was noted and the absorbance of the carotene extract was measured in a spectrophotometer (Beckman DU64) using extinction coefficient $E^{1\,cm}_{1\%}$ in petroleum ether, beta carotene 2500 at 450 nm and petroleum ether as blank.

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Beta carotene : Beta carotene was analysed by the procedure of Nelis and Deleenheer (1983). The chromatographic system was that of Shimadzu model LC6A consisting of pump, controller SCL6A; a variable wavelength detector, SPD10AV; an integrator CR6A chromatopack and a stainless steel 25 cm x 4.6 mm separation column (Shimpack ODS 18, 5 µm particle size). Separation of provitamin A / beta carotene was achieved in an isocratic system using HPLC grade acetonitrile / dichloromethane / methanol as mobile phase at a flow rate of 2 ml/min. The detector wavelength was set at 450 nm and 20 µl of sample extract was injected onto the column. Solvents and samples were filtered through 0.45 µ membrane filters prior to use in HPLC. The HPLC was calibrated by injecting 20 µl of beta carotene standards. An external standard of all trans beta carotene (Sigma Chemicals, St Louis, USA) was used. Peak identification was based on retention time and comparison with standard and co-chromatography. Each sample was injected in duplicate and the concentration determined from the calibration plot in which peak area was plotted against concentration (mg/ml). The detector responses were linear over the concentration range of 25 to 100 ng per 20 µl. All results were expressed as micrograms per 100 g of fresh weight of the samples.

The total and beta carotene, (pro-vitamin A) contents of the selected raw green leafy vegetables are given in Table 1

A wide variation was observed in the carotene contents of the green leafy vegetables studied. In general, the total carotene contents ranged from 14,025 to 41,984 mcg/100 g and beta carotene ranged from 7,130 to 28,160 mcg/100 g of the fresh leaves. Of the ten GLV analysed in its raw form, drumstick leaves had the highest contents of total carotenes, followed by *agathi, araikeerai, mullakeerai* and others. With regard to beta carotene also, drumstick leaves showed the maximum values, followed by *araikeerai, ponnanganni, agathi* and others. Drumstick leaves, *araikeerai, agathi* and *manathakkali* leaves were found to be the best sources of pro-vitamin A. Except spinach and amaranth (tender), all other eight green leaves studied had more than 10,000 mcg of beta carotene. While the pro-vitamin A values obtained in the present study were comparable to ICMR (Gopalan et al 1996) values available for six greens, the beta carotene contents for drumstick leaves and *ponnanganni* were higher than those reported by ICMR. This may be attributed to seasonal variations, varietal differences, degree of maturity etc., (Rodriguez-Amaya 1997). In the green leafy vegetables studied, 40.1 to 85.3% of the total carotenes were beta carotenes indicating that the latter was the major carotenoid pigment. According to Narasinga Rao (1991), green leafy

TABLE 1. TOTAL AND BETA CAROTENE CONTENT OF SELECTED GREEN LEAFY VEGETABLES

Common name	Botanical name	Total carotenes, mcg/100g	Beta carotene, mcg/100g	Beta carotene, as% of total carotenes
Agathi	*Sesbania grandiflora*	39,735 (45,000)	16,590 (15,540)	41.8 (34)
Drumstick leaves	*Moringa oleifera*	41,984 (42,000)	28,160 (19,690)	67.0 (47)
Fenugreek leaves	*Trigonella Foenum graecum*	14,025 (11,800)	11,965 (9,100)	85.3 (77)
Mannathakkali Leaves	*Solanum nigrum*	32,290 (ND)	15,266 (ND)	47.3 (ND)
Paruppu keerai	*Portulaca oleracea*	17,520 (ND)	14,325 (ND)	81.8 (ND)
Amaranth, tender	*Amaranthus gangeticus*	20,013 (20,160)	8,040 (8,340)	40.17 (41)
Mullakeerai	*Amaranthus spinosus*	33,642 (ND)	13,941 (ND)	41.4 (ND)
Araikeerai	*Amaranthus tristis*	38,886 (ND)	25,212 (ND)	64.8 (ND)
Ponnanganni	*Alternanthera sessilis*	26,550 (24,000)	17,394 (5,440)	65.5 (23)
Spinach	*Spinacea oleracea*	10,325 (9,440)	7,130 (6,460)	69.1 (68)

Figures in parenthesis indicate reported the value of ICMR (Gopalan et al 1996)
ND - Not detected

TABLE 2. TOTAL AND BETA CAROTENE CONTENT OF SELECTED VEGETABLES AND FRUITS

Common name	Scientific name	Total carotenes, mcg/100g	Beta carotene, mcg/100g	Percent
Vegetables				
Carrot	*Daucus carota*	6,657 (8,840)	5,846 (6,460)	87.8 (73.1)
Pumpkin	*Cucurbita maxima*	3,008 (2,100)	1,998 (1,160)	66.4 (55.2)
Yam, elephant	*Amorphophallus ampanulatus*	1,626 (ND)	1,260 (ND)	77.5 (ND)
Sweet potato (white fleshed)	*Ipomoea batatas*	517 (ND)	298 (ND)	57.7 (ND)
Chillies, green	*Capsicum annum*	2,835 (2,430)	1,045.9 (1,007)	36.9 (41.4)
Fruits				
Papaya, ripe	*Carica papaya*	2,464 (2,740)	868 (880)	35.2 (32.1)
Mango	*Mangifera indica*	2.529 (2,210)	1,866 (1,900)	73.8 (90.0)
Orange	*Citrus aurantium*	1,192 (2,240)	184.3 (190)	15.4 (8.4)
Tomato, ripe	*Lycopersicon esculentum*	2,556 (3,010)	686 (590)	26.8 (19.6)
Banana, nendram	*Musa paradisiaca*	424 (ND)	310 (ND)	73.2 (ND)

Figures in parenthesis indicate reported value of ICMR (Gopalan et al 1996)
ND - Not detected

vegetables have the highest contents of beta carotene in the range of 80 to 96% of total carotenes.

The total and beta carotene contents of the selected vegetables and fruits are given in Table 2.

Vegetables evaluated were those which contribute significantly in the Indian dietaries, containing appreciable amounts of pro-vitamin A. In their raw forms, the highest total carotene was found in carrots (6,657 mcg/100 g), followed by pumpkin, green chillies, yam and sweet potato. With regard to beta carotene also, the highest contents were found in carrots (5,846 mcg/100 g), followed by pumpkin, yam, green chillies and sweet potato. When compared to ICMR values available for three vegetables, the study value for carrots was less than that reported by Gopalan et al (1996). Studies conducted by Bhaskarachary et al (1995 a) showed that the beta carotene value of carrots may range from 6500 ± 1500 mcg. For pumpkin and green chillies, the study values were higher than the ICMR reported values. The beta carotene value reported for yellow variety of sweet potato in the Philippines by Almeida and Pentedo (1992) showed a relative higher value of 540 mcg/100 g which could be probably due to varietal differences. With regard to the percentage of beta carotene in total carotenes, maximum percentage was observed in carrots (87.8) and least in green chillies (36.9). For the other three vegetables, the percentage of beta carotene ranged from 57 to 77%.

Vitamin A activity of fruits is generally lower than leafy vegetables and they have a complex mixture of carotene hydrocarbons and oxygenated derivatives, (Tee and Lim 1991). Mango and papaya contribute maximum beta carotene among fruits grown in India. The total carotene contents of tomato, mango and papaya were relatively higher in the order of 2,500 mcg/100 g. But the beta carotene value was maximum in mango (1,866 mcg/100 g), followed by papaya, tomato and others. In general, mango contained maximum carotenes and banana (*'nendran'*) contained least carotenes. A value of 360 mcg/100 g of beta carotene in banana was reported in the Philippines study (Wasantwisut and Attig 1995). In the present study, 73% of total carotene in mango was beta carotene, while in papaya, beta carotene content was 35%. The rest of carotenes in papaya may be beta cryptoxanthine (Bhaskarachary et al 1995 b). Except papaya, the percentage of beta carotene in total carotene was found to be lower than the ICMR reported values. The variations may be due to varietal differences, level of ripeness, seasonal and agroclimatic differences.

Table 3 gives the total and beta carotene contents of

TABLE 3. TOTAL AND BETA CAROTENE CONTENT OF GREEN LEAFY VEGETABLES COOKED IN *PORIYAL* FORM

Greens	Total carotenes, mcg/100g	Beta carotene, mcg/100g	Percentage loss	
			Total carotene	Beta carotene
Agathi	37,808			
Temperature	80°C	15845	4.8	4.5
Time	12 min			
Drumstick leaves	22,752			
Temperature	80°C	16194	45.8	42.5
Time	15 min			
Fenugreek leaves	13,911			
Temperature	78°C	10040	0.8	16.1
Time	10 min			
Manathakkali	30,730			
Temperature	75°C	13142	4.8	13.9
Time	10 min			
Paruppu keerai	16,040			
Temperature	78°C	12105	8.4	15.5
Time	10 min			
Amaranth, tender	19,072			
Temperature	79°C	7840	4.7	2.5
Time	10 min			
Mullakeerai	30,113			
Temperature	75°C	12110	10.5	13.1
Time	10 min			
Araikeerai	27,016			
Temperature	79°C	12771	30.5	49.3
Time	10 min			
Ponnanganni	23,016			
Temperature	79°C	15297	13.3	12.1
Time	10 min			
Spinach	8,729			
Temperature	79°C	5182	15.5	27.3
Time	10 min			

the green leafy vegetables cooked in *poriyal* form.

Of the ten greens analysed in *poriyal* form, agathi showed maximum total carotene (37,808 mcg) and drumstick leaves showed maximum beta carotene (16,194 mcg). In cooked forms, the percent loss of total carotene was in the range of 4.7 to 45.8 and beta carotene in the range of 2.5 to 42.5. The loss of carotenes could be attributed to oxidation and heat treatment. Speek et al (1988) showed 14 to 60% loss in vitamin A activity due to processing of Thai vegetables. According to Rahman et al (1990), *lal sak* and *mula sak* showed a loss of 31 and 40% respectively in cooked forms. In spite of the losses, it is evident that all the greens studied had more than 5000 mcg per 100 g of beta carotene in cooked form. For infants 1 to 1.5 tsp, for children < 1 tbsp and for adults 1 to 2.5 tbsp of greens daily would meet the RDA for vitamin A.

Table 4 gives the mean absorption of total and beta carotene from selected foods in adults.

The mean percentage absorption of total carotene was found to be maximum in papaya (86.0 %), followed by *mullakeerai* (53.4%), drumstick leaves (51.4%), and carrot (46.4%). The mean percentage absorption of beta carotene was found to be maximum in papaya (88.7 %), followed by drumstick leaves (66.8%), *mullakeerai* (55.9%) and carrot (55.3%). In general, beta carotene absorption was better than total carotene in the range of 55.3 to 88.7%.

The results of this investigation have revealed that inexpensive, locally available green leafy vegetables are the richest sources of beta carotene. From the provitamin A data, it is clear that 10 - 35 g of green leafy vegetables would be sufficient to meet the dietary beta carotene requirements of

TABLE 4 MEAN ABSORPTION OF CAROTENE FROM SELECTED FOODS IN ADULTS

Foodstuff	Total carotenes, mcg/100g		Percentage absorption	Beta carotene, mcg/100g		Percentage adsorption
	Intake	Excretion		Intake	Excretion	
Drumstick leaves	3412.8	1658.1	51.4	2429.2	794.4	66.8
Mullakeerai	4200.4	1957.6	53.4	2450.0	1078.7	55.9
Carrot	3749.5	1998	46.4	2425.7	1083.1	55.3
Papaya	6630.0	926.68	86.0	2470.0	277.9	88.7

1,600 and 2.400 mcg/day in pre-school children and adults, respectively. Further studies on oxygenated derivatives of carotenoids of vegetables and fruits are recommended.

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RESEARCH NOTE

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Occurrence of *Listeria* spp. in Processed Fishery Products

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Processed fishery products viz. frozen shrimps, frozen squids, frozen fish, minced meat, surimi, breaded and battered shrimps and smoked fish obtained from the seafood processing plants and fish markets located in Mangalore were investigated for the prevalence of *Listeria* spp. Among the 72 frozen seafood samples examined, 11.1 % were found to contain *Listeria* spp. The incidence of *Listeria* spp. in shellfishes, particularly shrimps was very high (14.3 %), when compared to its incidence in finfishes (4.3 %). The positive isolates were identified as *L. monocytogenes* and *L. innocua*. The incidence of *Listeria* was high in headless (HL) shrimps (44.4%), followed by peeled and undeveined (PUD) shrimps (23.1%). Of the 22 hot smoked fish samples consisting mainly of smoked tuna (masmin), 45.5% contained *L. monocytogenes*. The higher incidence of *L. monocytogenes* in the smoked fishery products suggested that more care had to be taken to prevent the post-process contamination in these products when compared to frozen fishery products, as otherwise these seafoods may pose a vehicle of infection to humans on consumption.

Keywords : *Listeria monocytogenes*, Processed fishery products, Frozen shrimps, Smoked fish, Masmin.

Seafoods are gaining popularity and ready-to-eat seafood items are increasing in the market, especially in the developed countries and simultaneously, the importance of the problems associated with *Listeria* spp. in seafoods is also increasing. Seafoods have been implicated in several sporadic cases of listeriosis (Facinelli et al. 1989; Frediksen 1991; Baker et al. 1993) and in two outbreaks (Lennon et al. 1984; Riedo et al. 1990) in the developed countries, which are the major importing countries of Indian seafoods. India is earning a huge sum of foreign exchange through the export of fish and fishery products. *L. monocytogenes* ranked fifth on the descending cost scale due to foodborne illness caused by bacteria in the USA (Zottola and Smith 1991). The USFDA regulations insist the absence of *L. monocytogenes* in ready-to-eat foods, including cooked and smoked seafoods (Fuchs and Reilly 1992).

Several studies involving various other foods have shown that *L. monocytogenes* can survive freezing and frozen storage. Weagant et al (1988) examined both the domestic and imported frozen raw seafoods in the USA and found *L. innocua* in 44% and *L. monocytogenes* in 28% of the samples. Noah et al (1991) reported that approximately 49% of the raw frozen seafood samples tested were positive for *Listeria* spp. Jemmi (1990) isolated *L. monocytogenes* from 9% of hot smoked samples and 14% of cold smoked samples. The fact that *L. monocytogenes* can occur in smoked fish led the British Government to consider banning the sale of smoked salmon by mail order companies. Dillon et al. (1992) detected *Listeria* in 11.3% of the smoked seafood products.

Frozen seafoods such as shrimps, squids, fish, minced meat, surimi, breaded and battered shrimps obtained from seafood processing plants located in and around Mangalore and smoked seafoods procured from Mangalore fish market were brought to the laboratory in sterile plastic bags and tested for the presence of *Listeria* spp. without further delay. The protocol followed for the isolation of *Listeria* spp. was adopted from USDA-FSIS (McClain and Lee 1988), USFDA (Lovett et al. 1987) and NGFIS (van Netten et al. 1989) methods.

Briefly, 25 g of sample was homogenised with 225 ml of Listeria pre-enrichment broth and incubated at room temperature (28+2^{0}C) for 24 h. Ten ml of pre-enriched sample was then transferred to 90 ml of USDA Listeria enrichment broth (UVM I) and incubated at room temperature for 24 h for primary enrichment. Then, 0.1 ml of the UVM I enriched sample was transferred to 10 ml of Modified Fraser Broth (MFB) and incubated at 37^{0}C for 18 to 24 h for secondary enrichment. The secondary enriched sample was finally streaked onto PALCAM (Polymyxin Aesculin Lithium chloride Ceftazidime Acriflavin Mannitol) agar plates and incubated at 37^{0}C for 48 h. Typical colonies were picked up and purified onto Tryticase Soy Agar (TSA) with 0.6 % yeast extract (YE) plates. They were further subjected to various biochemical test for characterization and identification (Jones and Seeliger 1992).

Of the 72 frozen seafood samples tested, 8 samples (11.1%) were found to contain *Listeria* spp. (Table 1). Among the 23 frozen finfish samples, only one surimi sample contained *Listeria* spp. which was identified as *L. monocytogenes*. None of the other samples contained *Listeria* spp. On the contrary, the incidence of *Listeria* spp in frozen shellfish samples was fairly high (14.3%), when compared to its incidence in finfishes (4.3%). The reason for the higher incidental rate of *Listeria* spp. in shellfishes could not be clearly understood, because of its ubiquitous nature. However, it is believed that shellfishes provide a more ideal environment for the growth of *Listeria* spp. than finfishes. *Listeria* spp. were detected in frozen shrimps, but not in any of the frozen squids. In shrimps, the incidental rate of *Listeria* spp. was higher in frozen headless (HL) shrimps (44.4%) than in peeled and undeveined (PUD) shrimps (23.1%), which may be because of the peeling process that might have reduced contamination. *Listeria* spp. were totally absent in the peeled and deveined (PD) shrimps and in breaded and battered shrimps. The reason could be that

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TABLE 1. INCIDENCE OF *LISTERIA* SPP. IN FROZEN SEAFOODS

Type of sample	No. of samples tested	Number of samples positive for *Listeria* spp.	*L. monocytogenes*	*L. innocua*
Finfishes	23	1 (4.3%)	1 (4.3%)	-
Whole fish	1	-	-	-
Minced meat	5	-	-	-
Surimi	17	1	1	-
Shellfishes	49	7 (14.3%)	4 (8.2%)	3 (6.1%)
Shrimps	35	7 (20.0%)	4 (11.4%)	3 (8.6%)
Headless (HL)	9	4	3	1
Peeled and undeveined (PUD)	13	3	1	2
Peeled and deveined (PD)	3	-	-	-
Breaded and battered	5	-	-	-
Breaded, battered and fried	5	-	-	-
Squids	14	-	-	-
Whole	2	-	-	-
Tubes	7	-	-	-
Tentacles	5	-	-	-
Total	72	8 (11.1%)	5 (6.9%)	3 (4.2%)

most of the breaded and battered shrimps are prepared out of PD shrimps, in which the gut contents are removed along with the vein and cleaned to prevent harbouring of microorganisms.

Of the 8 *Listeria* spp. isolated from frozen fishery products, 5 were identified as *L. monocytogenes* and 3 as *L. innocua*. The incidental rate of *L. monocytogenes* was generally higher (6.9%) in the frozen fishery products than *L. innocua* (4.2%). Very few studies have been carried out in India regarding the prevalence of *Listeria* spp. in processed seafoods. These preliminary studies (Fuchs and Surendran 1989; Manoj et al. 1991; Kamat and Nair 1994) have indicated that *L. monocytogenes* was totally absent in seafoods so far analysed and only *L. innocua* and *L. grayi* were present. Considering the importance of *L. monocytogenes* as a human pathogen and its ubiquitous nature, this study was undertaken to further explore the prevalence of *L. monocytogenes* in the processed Indian seafoods. Since the habitat of all the species of *Listeria* is same and when some *Listeria* spp. are detected in the samples, there is a possibility for the occurrence of the pathogenic *L. monocytogenes*. The incidence of *Listeria* spp. detected in this study was lower than that reported by Weagant et al (1988), who found that 44% of both the domestic and imported frozen raw seafoods contained *L. innocua* and *L. monocytogenes*. However, Kvenberg (1988) reported that only 5 to 6 % of imported and domestic seafoods in the USA were positive for *L. monocytogenes*. Fuchs and Surendran (1989) isolated *L. innocua* from 36% of frozen raw fish samples tested in Cochin. About 10.5% of frozen seafood samples in Taiwan were found to contain *L. monocytogenes* (Wong et al. 1990). Manoj et al (1991) isolated *L. innocua* from frozen shrimps of Mangalore. The presence of *L. monocytogenes* in raw frozen shrimp samples was reported as 9% by Hartemink and Georgsson (1991). However, Noah et al (1991) isolated *Listeria* spp. from 49% of raw frozen seafood samples examined. Kamat and Nair (1994) isolated only *L. grayi* (60%) and *L. innocua* (40%) from frozen shrimp samples analysed in Bombay. Varying incidental rates for *L. monocytogenes* reported by different workers may be due to the differences in the extent of contamination or due to the differences in the isolation methodologies.

The incidence of *Listeria* spp. in smoked seafoods is presented in Table 2. Of the 22 hot smoked fish samples which consists mainly masmin, a traditional smoked product prepared from tuna, 10 samples were found to contain *L. monocytogenes*. Other species of *Listeria* were not isolated from smoked seafoods. Smoked seerfish and smoked fish cake were free from *Listeria* spp. The incidental rate of *Listeria* spp. was found to be quite higher in smoked seafoods when compared to frozen fishery products. Results show that 45.5% of the smoked seafood samples tested were positive for *Listeria* spp. Only *L. monocytogenes* was isolated from the smoked seafoods, which is in line with the observation of other workers. Jemmi (1990) reported that 24% of smoked seafood samples were positive for *L. monocytogenes*. Hudson et al (1992) reported that 35.7% of smoked mussels and 75% of smoked salmon samples examined were positive for *L. monocytogenes*. *Listeria* spp. were isolated from 16.7% of smoked fish products examined (Dillon et al. 1994), with hot smoked products yielding 25.4 % of the isolates. The smoked seafoods tested in this study mainly belonged to only one species i.e. smoked tuna. The incidence of *L. monocytogenes* in these salted, cooked, dried and hot smoked fish fillets could be because the varying processes applied by the traditional

TABLE 2. OCCURRENCE OF *LISTERIA* SPP. IN SMOKED SEAFOODS

Type of sample	No. of samples tested	Number of samples positive for *Listeria* spp.	*L. monocytogenes*
Smoked tuna	20	10 (50.0%)	10 (50.0%)
Smoked seerfish	1	-	-
Smoked fishcake	1	-	-
Total	22	10 (45.5%)	10 (45.5%)

manufacturers and they seldom follow the standard hot smoking protocol, as these processes should have been sufficient to be listericidal, if applied properly. Another important reason could be post-process contamination and/or contamination during sun drying, as invariably these products are prepared near the sea shore in open condition. The presence of *L. monocytogenes* in these products are very dangerous as these products are very popular in certain parts of Indian and are also exported to Southeast Asia. These products are used in the preparation of various traditional dishes and some of the dishes like "Chutney" are consumed without further cooking (Nair et al. 1994), which may pose a health hazard for the consumers who consume these products contaminated with *L. monocytogenes*.

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RESEARCH NOTE

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Effect of Chilling and Freezing on the Survival of *Listeria monocytogenes* in Shrimps

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The effect of chilling and freezing on the survival and growth of *Listeria monocytogenes* in shrimps was investigated. Fresh shrimps (*Parapaeneopsis stylifera* and *Metapenaeus dobsonl*) were beheaded and inoculated with *L. monocytogenes* (NCTC 1994) at a level of 10^5 cfu/g. The inoculated shrimps were stored at the refrigerated (4-7^{0}C) and frozen conditions (-18 to - 20^{0}C), respectively. A log increase in counts of *L. monocytogenes* was observed in chilled shrimps by the 2nd day of storage and thereafter the counts remained almost same throughout the storage period of 12 days. The reduction in counts of *L. monocytogenes* was drastic in shrimps during the first day of frozen storage. Though the listerial load almost remained constant during the initial 30 days of frozen storage, it nearly doubled by the 60th day and came down to the earlier level by the 90th day. The results suggested that *L. monocytogenes* in shrimps was not completely killed by the process of chilling and freezing.

Keywords : *Listeria monocytogenes*, Chilling, Freezing, Growth, Survival, Shrimps.

Listeria monocytogenes has been recognised as an important food-borne pathogen since 1981 and seafoods have been implicated in several sporadic cases of listeriosis (Facinelli et al 1989; Frediksen 1991; Baker et al 1993) and in two outbreaks (Lennon et al. 1984; Riedo et al. 1990). Buchanan et al. (1989) isolated *L. monocytogenes* from 15% of uncooked seafood samples. Motes (1991) found that approximately 11% of the shrimp samples were positive for *L. monocytogenes*. Examination of both the domestic and imported frozen raw seafoods in the U.S.A revealed that *L. monocytogenes* was present in 28% of the samples (Weagant et al. 1983). *L. monocytogenes* has also been isolated from 9% of raw frozen shrimp samples by Hartemink and Georgsson (1991). *L. monocytogenes* grew well on crabmeat during refrigeration (Brackett and Beuchat 1990). Lovett et al (1990) reported that there was an increase in the load of *L. monocytogenes* in shrimps held at 7^{0}C. Farber (1991) demonstrated that the growth of *L. monocytogenes* at 4^{0}C occurred on artificially inoculated shrimps. Harrison et al (1991) studied the fate of *L. monocytogenes* on frozen seafoods and found that population on frozen shrimps decreased by less than one log after 3 months of storage at - 20^{0}C. Golden et al (1988) found that this bacterium is relatively resistant to damage due to freezing. Walker et al (1990) reported the growth of *L. monocytogenes* at refrigeration temperatures. Against this background, the effect of chilled and frozen storage temperature on the survival and growth of *L. monocytogenes* in shrimps was investigated.

Fresh shrimps (*Parapenaeopsis stylifera* and *Metapenaeus dobsonf*) procured from fish market of Mangalore, India were washed, beheaded and kept in ice till they were used for the inoculation studies. They were initially tested for the presence of listeriae. The *Listeria monocytogenes* (NCTC 1994) bath was prepared by growing the culture overnight at 37^{0}C in 300 ml trypticase soya broth (TSB) containing 0.6% yeast extract (YE) and centrifuging at 5000 rpm for 10 min. at 4^{0}C. The cell pellet was washed twice in sterile physiological saline and suspended in 5 ml saline and the whole cell suspension was added to 1 litre sterile saline and the load of listeriae was tested immediately after the addition of *L. monocytogenes* by plating serial dilutions onto modified McBride's Listeria (MML) agar plates and incubating at 37^{0}C for 24 to 48 hr. Typical colonies were confirmed by gram reaction, catalase and motility tests (Jones and Seeliger 1992).

The headless shrimps (*P. stylifera* and *M. dobsonf*) were separately immersed in the inoculation bath for 1 min. and then drained. The first set of the inoculated shrimps (*P. stylifera*) was stored at refrigerated temperatures (4-7^{0}C) and the second set of inoculated shrimps (*M. dobsoni*) was stored at frozen storage temperatures (- 18 to - 20^{0}C). The listerial load on the treated shrimps was determined immediately after the dip treatment by drawing the samples in triplicates. The survival and growth of *L. monocytogenes* on chilled shrimps and frozen shrimps were studied separately by analysing the listerial load at different time intervals during refrigerated and frozen storages, respectively.

The shrimp samples were initially free of listeriae. After experimental inoculation, they were found to have *Listeria monocytogenes* counts of 1.04×10^5 cfu/g and 2.50×10^5 cfu/g, respectively (Tables 1 and 2). The listerial count in chilled shrimps was found to increase by a log on the 2nd day of refrigerated storage (Table 1) and thereafter the counts remained almost same throughout the storage period of 12 days. On the other hand, the listerial count on the frozen shrimps was found to decrease drastically during the first day of frozen storage (Table 2). The load on the frozen shrimps remained constant during the initial 30 days of frozen storage. But on the 60th day, the listerial counts had nearly doubled. However, on the 90th day, the counts again came down to the earlier level.

The results showed that *L. monocytogenes* can survive and grow in shrimps under refrigerated condition (Table 1).

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TABLE 1. SURVIVAL OF *LISTERIA MONOCYTOGENES* IN CHILLED SHRIMPS (*PARAPAENEOPSIS STYLIFERA*)

Period, days	*L. monocytogenes*, cfu/g
0	$1.04 \pm 0.12 \times 10^5$
1	$8.76 \pm 0.25 \times 10^5$
2	$2.12 \pm 0.14 \times 10^6$
3	$2.80 \pm 0.19 \times 10^6$
4	$3.32 \pm 0.37 \times 10^6$
5	$2.82 \pm 0.24 \times 10^6$
6	$7.72 \pm 0.62 \times 10^6$
7	$8.80 \pm 0.51 \times 10^6$
8	$6.80 \pm 0.49 \times 10^6$
9	$4.52 \pm 0.09 \times 10^6$
10	$9.84 \pm 0.53 \times 10^6$
11	$3.94 \pm 0.23 \times 10^6$
12	$2.32 \pm 0.34 \times 10^6$
13	*DC

*DC - Discontinued

TABLES 2. SURVIVAL OF *LISTERIA MONOCYTOGENES* IN FROZEN SHRIMPS (*METAPENAEUS DOBSONI*)

Period, days	*L. monocytogenes*, cfu/g
0	$2.50 \pm 0.18 \times 10^5$
1	$9.12 \pm 0.13 \times 10^4$
3	$6.00 \pm 0.24 \times 10^4$
7	$6.92 \pm 0.29 \times 10^4$
14	$4.36 \pm 0.21 \times 10^4$
21	$4.72 \pm 0.32 \times 10^4$
30	$6.08 \pm 0.30 \times 10^4$
60	$1.14 \pm 0.43 \times 10^5$
90	$4.55 \pm 0.41 \times 10^4$

Though on the 2nd day of storage, one log rise in counts was noticed, the counts remained constant during the latter period of chilled storage, till the shrimps were spoiled. It has been reported that *L. monocytogenes* was able to proliferate in inoculated raw oysters (Cassiday et al. 1989) and fresh crab meat (Hitchins and Tran 1990). The results are more or less in confirmation with the findings of Lovett et al (1990) who reported that there was an increase in listerial counts when *L. monocytogenes* was artificially inoculated into shrimps and crab meat, and stored at 7^0C for 14 days. In this study, the storage time was 12 days and the initial numbers were high. Nevertheless, there was 1-2 log unit increase in counts during storage occurred. Brackett and Beuchat (1990) found a 2-5 log increase in listerial load, when *L. monocytogenes* was inoculated into crab meat and held at 5^0C and 10^0C for two weeks. Farber (1991) also reported that *L. monocytogenes* grew fairly well on lobsters and shrimps and in most cases increasing about 2-3 logs within 7 days at 4^0C. The results of the present study suggest that low numbers of *L. monocytogenes* present in raw seafoods might multiply and reach dangerous levels during storage at refrigerated temperatures.

Results showed that *L. monocytogenes* populations almost remained constant in frozen shrimps during the first 30 days of storage at frozen temperature, despite a decrease in one log cells during freezing (Table 2). During the 60th day, the listerial counts were double of what was observed on the 30th day. This apparent increase was only an experimental artefact and it might be due to variations in the level of contamination of individual shrimps. However, on the 90th day, the counts came down to the earlier constant level. The findings support the statement that freezing and frozen storage have no significant effect on *L. monocytogenes* (Sneath et al. 1986) and this organism is relatively resistant to damage due to freezing (Olsen et al. 1988). McCarthy et al (1990) also recovered *L. monocytogenes* from laboratory contaminated raw shrimps after storage at -20^0C for upto 90 days. The findings of Harrison et al (1991) that *L. monocytogenes* populations on frozen shrimps decreased by less than one log after 3 months of storage at -20^0C also supported the results of the present study.

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SAFETY EVALUATION OF CERTAIN FOOD ADDITIVES AND CONTAMINANTS : WHO Food Additive Series - 46. Prepared by the fifty fifth meeting of the Joint FAO / WHO Expert Committee on Food Additives (JECFA). Published by World Health Organization, Geneva. 2001, pp 392. Price Sw. fr. 80 (Price in developing countries Sw.fr. 56

The monographs contained in the volume were prepared at the fifty fifth meeting of the Joint FAO / WHO Expert Committee on Food Additives (JECFA) which met at WHO headquarters, Geneva,Switzerland, 6 - 15 June 2000. These monographs summarise the safety data on selective food additives and contaminants as follows:

Flavouring agent: Furfural, Furfuryl alcohol, Cinnamyl alcohol, Phenol, Pulegone and related flavouring agents, Food colour : Cochineal extract, caramines and carmnic acid and caramel colour - II. Sweetening agent : D-tagatose and trehalose. Contaminants : Cadmium and tin.

Drafts for individual groups of additives have been prepared separately by the experts in the field and are based upon the ongoing research work papers prepared by them.Each monograph is presented following the sequence of explanation - citing brief information regarding the safety of additives and about the previous meeting biological data covering biochemical aspects such as absorption, distribution and excretion in animals / humans (in some cases). Biotransformation and toxicological studies involving acute-short term-long term toxicity studies, carcinogenicity studies and special studies on genotoxicity. All these are expressed in tables followed by comments,evaluation and references with relevant background information.

With regard to food colours and sweetening agents the committee considered an ADI : Not specified, but indicated an alert of individuals who are allergic to the colours and recommended for further evaluation of D - tagatose and trehalose.

Five groups of flavouring agents were evaluated using procedure for the safety of flavouring agent, in applying the procedure, the committee has noted that first, the substance is assigned to a structure class, secondly the key element of the procedure involved in determining whether a flavouring agent and products of its metabolism are innoccuos and / or endogenous substance. Finally, the intake threshold among is considered to know as to whether additional data are required or not.

Applying the procedure and also referring some toxicological report the committee evaluated furfural, furfuryl alcohol, cinnamyl alcohol, phenol, pulegone and related flavouring agents.

Furfural and furfural alcohol : The Committee established a group of 0-0.5 mg/kg body weight for furfural and furfural alcohol on the basis of the NOEL of 53 mg/kg bw per day in 13 weeks study in rats and a safety factor of 100 was applied. Other flavouring agents in this group are in the range of 0.003-690 (g/day).

Regarding phenol and its derivatives, the Committee reviewed a wide range of studies on animals that provided qualitative and quantitative information, on toxicological studies and on special studies. The Committee recommended an ADI of 0-0.4 mg/kg bw (FAO 1999) for 2-phenyl phenol.

The Committee established a NOEL of 0.44 mg/kg bw per day for Pulegone and related flavouring substances in a 90 days studies is a >1000 times the estimated daily intake of isopulegone.

Contaminants : Cadmium : The cadmium studies have several noteworthy features that make it valuable source of data on health effects. Even though there is a risk for tubular dysfunction when exposed at the PTWI of 7 μg/kg bw the committee considered this value because of inprecise risk estimation. Hence the Committee recommended the area of investigation on various Biochemical and toxicological aspects.

Tin : The committee considered by intake of tin of the several countries do not exceed the PTWI of 14 mg/kg bw. However, at fifty-fifth meeting because of insufficient data Committee could not establish acute reference dose for tin. The concentration of tin in food in various forms are presented in appendix I, appendix II and appendix III.

The volume has 4 annexures that give 1) Report of the previous meeting of JFCFA 2) Abbreviation used in monograph 3) Members and secretariat name of the participants 4) Acceptable daily intake, other toxicological information on specification of various food additives and contaminants.

The primary strength of this book is that it provides a comprehensive review of diverse subject areas, many of which will be beyond any individual expertise, but potentially impact upon both direction and approach of present and future research, the primary target audience would be scientist or students whose focus is evaluation research on food additives. This is a very informative book concerning issue which affect anyone even remotely involved with food additive, like in food industries, Universities, Research institutes. Standard of editing is extremely high and cross referring of chapters is good. It not only provides a scientific basis for safety evaluation but also assesses possible risk. In summary the book gives not only suggestions on food additives, but also provides updated literature review on particular issues. The book can be recommended as a valuable reference tool for researchers and graduate students in food science and nutrition.

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EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD : Fifty-fourth Report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series, No. 900. WHO, Geneva 2001, pp. vi+96, ISBN 92 4 120900 3, Price : Sw.fr. 20, Price in developing countries : Sw.fr. 14.

This report presents the conclusions of fifty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives convened in Geneva from 15 to 24 February 2000. The Committee's purpose was to provide guidance to FAO and WHO Member States and to the Codex Alimentarius Commission on public health issues pertaining to residues of veterinary drugs in foods of animal origin. The specific tasks before the Committee were to evaluate the safety of residues of certain veterinary drugs in food, and to establish Acceptable Daily Intakes (ADIs) and recommend Maximum Residue Limits (MRLs) for such drugs.

The book comprises of five sections. Section 1 gives brief introduction to the background and purpose of the Meeting. Section 2 describes the Committee's recommendations regarding interpretation of data on inhibition of the activity of various cholinesterases, particularly brain and erythrocyte acetylcholinesterase and plasma and brain butyrylcholine esterase. It also includes the details of the recommendations arising from an informal meeting held in Rome in February 1999 to harmonize the work of the Committee and the Joint FAO/WHO Meeting on Pesticide Residues.

Section 3 constitutes the major part of the book. It deals with the evaluation of residues of some specific veterinary drugs and the recommendations made by the Committee. The drugs include one anthelminthic agent (ivermectin), four antimicrobial agents (flumequine, lincomycin, oxytetracycline, tilmicosin), six insecticides (cyhalothrin, cypermethrin, α-cypermethrin, dicyclanil, permethrin, metrifonate/trichlorfon) and one production aid (melengestrol acetate). The section describes systematically the results of a number of studies on the residues, toxicity, pharmacokinetics and metabolism of these drugs in food, animal species like cattle, sheep, pigs, chickens and trout. ADIs and MRLs for these drugs have been established based on the data of these studies. Short descriptions of the analytical methods have also been given.

The section begins with comments on ivermectin, a broad-spectrum antiparasitic drug used against nematodes and arthropods in food-producing animals. The Committee at its thirty-sixth and fortieth meetings previously reviewed the drug. At the latter meeting, the Committee allocated an ADI of 0-1 μg/kg of body weight and recommended MRLs of 100 μg/kg for liver and 40 μg/kg for fat in cattle. In the present report, a study on the secretion of its residues in milk of dairy cows on topical application has been described. Based on the study, the Committee recommended a temporary MRL of 10 μg/kg for whole milk in cattle.

Among the antimicrobial agents, the Committee evaluated lincomycin for the first time. Results of a large number of toxicological studies on lincomycin in dogs, rabbits, mice, rats, cats and human beings have been tersely described in the report. Data on pharmacokinetics and metabolism of the drug and residues in cows' milk and tissues of veal calves, pigs, sheep and chickens have also been presented. The Committee decided an ADI of 0-30 μg/kg of body weight for lincomycin. The recommendations of the Committee on MRLs for the drug in the edible tissues of cattle, sheep, pigs and chickens and cows' milk have also been made available in the report but the MRL for chickens' eggs could not be finalized due to the need of more data on this aspect. The recommended MRLs for lincomycin in pigs are 100 μg/kg for muscle and fat, 500 μg/kg for liver and 1500 μg/kg for kidney. The Committee recommended the same MRLs for edible tissues of cattle, sheep and chickens but made them temporary. An MRL of 150 μg/kg was recommended for cows' milk.

Flumequine was previously considered by the Committee at its forty-eighth meeting, when it established an ADI of 0-30 μg/kg of body weight and recommended MRLs in cattle and temporary MRLs in other animals. In the present report, the Committee examined more data on drug residues and decided MRLs of 500 μg/kg for muscle and liver, 3000 μg/kg for kidney and 1000 μg/kg for fat in cattle, pigs, sheep and chickens. The Committee also recommended an MRL of 500 μg/kg for trout muscle with skin in normal proportions.

The ADI and MRLs for oxytetracycline recommended by the Committee in its fiftieth meeting for tissues, milk and eggs have also been presented in this report. Similarly, the ADI and MRLs recommended for tilmicosin in the forty-seventh meeting have been retained in the present report but the temporary MRL for ewes' milk has not been extended.

The report includes elaborate account of studies on insecticides cyhalothrin, dicyclanil and metrifonate (trichlorphon) that have been considered by the Committee for the first time. For cyhalothrin, a temporary ADI of 0-2 μg/kg of body weight was established. The temporary MRLs in cattle, pigs and sheep constitute 20 μg/kg for muscle, liver and kidney, and 400 μg/kg for fat and 30 mg/kg for cows' milk. An ADI of 0-7 μg/kg of body weight was established for dicyclanil for MRLs of 200 μg/kg for muscle, 400 μg/kg for liver and kidney, and 150 μg/kg for fat in sheep were recommended. For metrifonate, an ADI of 0-20 mg/kg of body weight was established in the present report and MRL of 50 μg/kg was recommended for cows' milk.

Regarding permethrin, the Committee was of the opinion that the available toxicological database was inadequate to formulate recommendations on it. After reconsideration of cypermethrin and α-cypermethrin, the Committee decided not to extend the temporary MRLs recommended for these in forty-seventh meeting of the Committee.

Melengestrol acetate, which is used as a production aid to improve the efficiency of feed conversion, promote growth and suppress estrus in female beef cattle, was evaluated for the first time. The report carries detailed information on its pharmacokinetics, biotransformation, toxicity, carcinogenicity, genotoxicity, reproduction and developmental toxicity, and its

residual concentrations in edible tissues of beef cattle. An ADI of 0-0.03 µg/kg of body weight was established and the Committee recommended temporary MRLs of 2 µg/kg for liver and 5 µg/kg for fat in cattle, however, MRLs for muscle and kidney were not recommended, as the concentrations in these tissues are generally low.

The Committee's note on the need of future work regarding updating the risk assessment procedures has been included in section 4. Due to continuing need for evaluations of veterinary drugs, the Committee gave recommendations in section 5 to hold the meetings regularly. A list of 15 references has been given at the end of this section.

Three annexures containing valuable information have been appended at the end of the report. The Committee's recommendations on the ADIs and MRLs for each drug have been presented in easy to grasp tabular form in the Annexure 2. It may act as a ready reckoner for those concerned with food safety. A list of 145 reports and other documents resulting from the Committee's previous meetings on food additives has been given in Annexure 1. It may prove to be a useful reference tool for those interested in food additives. Annexure 3 includes the proposed draft definitions of meat, muscle and fat.

The document is a rich source of diverse and authentic information and is sure to be useful for the food industry, quality control laboratories, producers of veterinary drugs, scientists and academic institutions. The Committee and the World Health Organization have done a commendable job and deserve appreciation for bringing out this publication.

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ENVIRONMENTAL STRESSORS IN HEALTH AND DISEASE : Edited by Jurgen Fuchs and Lester Packer, Marcel Dekker Inc. 270, Madison Avenue, New York, NY 10016, 2001, Price US $ 195, pp 504

Compelling support for the involvement of free radicals in disease development comes from epidemiological studies showing that an enhanced antioxidant status is associated with reduced risk of several diseases. Elevated antioxidant status is also associated with decreased incidence of cataract and cancer. Lowering oxidative stress can have a clinical benefit. This book deals with the natural antioxidant enzymes and antioxidant substances such as Vitamins A, C, E, flavonoids, polyphenols, carotinoids, lipoic acid and other nutrients present in foods and beverages and highlights the role of the environment in oxidative processes involved in health and disease.

The free radicals and other reactive species are involved in different human diseases, implicated in over 100 disorders, ranging from scleroderma and hemorrhagic shock to cardiomyopathy and cystic fibrosis to gastrointestinal ischemia, AIDS, hearing loss and even male pattern baldness. Oxidative stress can result in adaptation of cell or organism by up regulation of defense systems that may (a) completely protect against damage, (b) protect against damage to some extent, but not completely and (c) overprotect. Progress in understanding the effects of treatment on oxidative damage in human disease will be greatly facilitated when biomakers of oxidative damage are routinely included in clinical trials of therapeutic agents.

Inflammation is the local physiological response to tissue injury, that can be initiated by a microbial infection, physical agent, irritant or corrosive chemical. There are two types; acute and chronic inflammation. Rheumatoid arthritis is an example of a chronic inflammatory joint disease, in which the exogenous antigen is unknown. An increase in free radical production is a necessary part of the inflammatory response. Reactive oxygen species include super oxide, hydrogen peroxide and hydroxyl radical.

Biologically, the most important antioxidants are glutathione, glutathione peroxidase, vitamin E and C, SOD, catalase and urate. Excessive free radical production causes the depletion of these antioxidant pools, leading to an imbalance in antioxidant / oxidant ratio and also cause damage to DNA, lipids, carbohydrates and proteins. Free radicals attack fatty acids present in cell membranes including linoleic acid, arachidonic acid, and other poly-unsaturated fatty acids.

It has been speculated that oxidation of many different cell components stimulates auto anti bodies. Ozone is an environmental source of oxidizing potential that is a byproduct of petroleum burning particularly as gasoline in motor vehicles. Ultraviolet light also mediates immuno-suppression. Concerning cell proliferation, the induction of oxidative stress has particularly profound effects on the tumor promotion stage of carcinogenesis. Oral exposure to arsenic occurs primarily from contamination of drinking water and food constituents. Exposure to inorganic arsenic is associated with increased risk of cancers of the skin and internal organs, including the urinary bladder, respiratory tract and liver. In stark contrast to the carcinogenic effects of chronic arsenic exposure, arsenic trioxide has been used therapeutically to successfully treat patients with acute promyelocytic leukemia without causing severe toxicity. To protect cells from damage, various defense mechanisms have been developed against oxidants. Cigarette smoking has been implicated as a significant contributing factor in the etiology of respiratory, cardiovascular and other disorders. Oxidative stress has been implicated in the pathogenesis of numerous diseases of the lung, including cystic fibrosis, chronic obstructive airway disease and asthma. Human smokers have lower blood vitamin C levels and decreased urinary excretion of vitamin C. Vitamin E is the most important fat-soluble antioxidant and free radical scavenger. Increased oxidative damage to DNA is associated with cigarette smoking. The nutritional status

of experimental subjects plays a role in modulating the action and metabolism of chemicals, drugs, and environmental agents.

In recent years, there has been a progressive increase in urban air pollution, characterized by high concentrations of atmospheric suspended particulate matter (SPM). Occupational exposures to asbestos fibers and crystalline silica particles are associated with the development of fibrotic lung disease or pulmonary fibrosis. Asbestosis and silicosis are distinct lesions histologically in the lung, but are fundamentally similar in their outcome, a build up of collagen and extra cellular matrix material that may progress to ventilatory and restrictive abnormalities with reduced diffusing capacity. In addition to causing pulmonary fibrosis, workers exposed to asbestos develop malignant mesotheliomas, non-malignant pleural diseases and lung cancer. Interactions between asbestos fibers and cigarette smoke increase risks of bronchogenic carcinoma and possibly pulmonary fibrosis may be dependent upon iron catalyzed free radicals. Administration of antioxidants may be a feasible approach clinically for prevention and/or therapy of asbestos and silica-induced lung diseases. Studies with blood plasma have indicated that exposure of plasma to O_3 or NO_2 results in rapid depletion of ascorbic and uric acid, suggesting that these antioxidants provide the major defense system against both of these oxidant gases.

Oxidative stress and inflammation have been linked to cutaneous damage in contact dermatitis. Free radical reactions are particularly significant for photoallergy reactions. Atopic dermatitis is a genetically determined T-cell mediated inflammatory skin disease. Parental smoking increased the prevalence and severity of the atopic state in children. Not everyone exposed to the chemicals will develop leukoderma, suggesting involvement of an individual genetic susceptibility. Occupational and environmental agents can cause or exacerbate acne and folliculitis. It remains to be seen whether antioxidants or antioxidant combinations will prove beneficial against distinct aspects of contact dermatitis in humans. Chromium is an essential trace element ubiquitous in the environment. In its hexavalent state, chromium is cytotoxic, genotoxic and carcinogenic in animals. Upon skin contact with cobalt metal, cobalt salts are readily formed in human skin and penetrates into the epidermis. Wilson's disease is an autosomal recessive form of chronic copper intoxication, caused by the lack of the copper carrier protein ceruloplasmin. The massive iron overload causes dysfunction of the liver, heart and pancreas finally leading to hepatic cirrhosis, liver cancer, diabetes and cardiac insufficiency. Nickel is an essential trace element present in most alloys used in the manufacture of common utensils, coins and casual skin contact is virtually unavoidable. Platinum and palladium are released into the environment by the widespread use and disposal of automobile catalytic converters and are considered as important environmental toxins. Occupational exposure to the essential trace element vanadium occurs in the mining and steel industry as well as in petroleum refining, while environmental contact is usually through fly ash. Aluminum is present abundantly in the earth's crust and industrial activities have led to a large mobilization of aluminum.

Chronic environmental arsenic poisoning is a serious problem in developing countries and caused by drinking water naturally contaminated with arsenic. Dietary intake as well as smoking of cigarettes is the primary source of human exposure to cadmium. Lead is the longest used and best recognized toxic environmental chemical. Although the skin is a rich sulfhydryl group organ, it is not a target organ of lead toxicity. Mercury exposure occurs through environmental contamination as the result of mining, smelting and industrial discharge. The major route of mercury exposure is dietary intake. It is clearly established that the mechanism of ethanol toxicity involves oxidative stress. Benzene is a major industrial chemical and gasoline additive and has myelotoxic and leukemogenic effects in humans. Naphthalene is a pulmonary, renal and ocular toxicant, and it also causes hemolytic anemia in humans. Nitrated polycyclic hydrocarbons are widely distributed in the environment mainly as the result of incomplete combustion process. Approximately one third of all pesticide-related diseases are dermatological. Organo-tin compounds readily penetrate through the skin and may cause systemic toxicity. Reactions among indoor pollutants can produce free radical species and reactive aldehydes, ketones, and peroxyacyl nitrates. Typical indoor air pollutants are the oxides of nitrogen, ozone, tobacco smoke, volatile organic peroxides, terpenes, and mineral fibers such as asbestos. Hydrofluric acid readily penetrates deep into the skin and subcutaneous tissues causing severe skin irritation and corrosion. White phosphorus burns causing deep thermal injuries in skin and underlying tissue and may result following spontaneous combustion when contaminated skin surfaces dry.

Solar ultraviolet radiation has long been known to have beneficial as well as deleterious effects on human skin. It also causes several types of photodermatosis, premature aging, cancer, immune suppression and cataract formation. Membranes are another possible cellular target responsible for the UVA-induced cellular lethality due to the presence of free radicals that can produce lipid per-oxidation and membrane damage. In the lung, ozone has been shown to exhibit toxic effect.

Direct corneal damage can occur since the cornea is most directly exposed to noxious influences of the environment. Organic solvents, acids and alkali can cause chemical burns. Smoke from tobacco or cooking fires carry increased free radicals that may help in the development of cataracts. Deficiencies of selenium and vitamin A and E also can result in retinal capillary and/or rod and cone photoreceptor cell damage. Oxidative damage and anti oxidant protection in ocular tissues is of increasing interest because the eye is exposed to and susceptible to sunlight, pollutants, oxygen and various chemicals. Vitamin C and E are often considered "gold standard" antioxidant vitamins since their function and importance in the maintenance of pro- and anti oxidative homeostatis is well characterized in a wide variety of biological systems. Age related macular degeneration would remain a major ocular public health problem for the foreseeable future.

The best recommendations for patients wishing to modify their risk of AMD is to quit smoking, avoid excessive sunlight and to consume a healthy diet rich in fruits and vegetables. Hopefully, in the near future, a clear consensus on rational, scientifically based lifestyle, phot-protective, nutritional and pharmacological interventions will emerge so that AMD can be prevented and excellent vision can be preserved for the vast majority of the elderly population. Assessing oxidative damage in human tissues and body fluids and the increasing cooperation between laboratories in exchanging samples is contributing towards the path of improvement.

These various aspects are covered in different chapters in the book. I recommend this book as a valuable source of information for Environmental stressors in Health and Disease.

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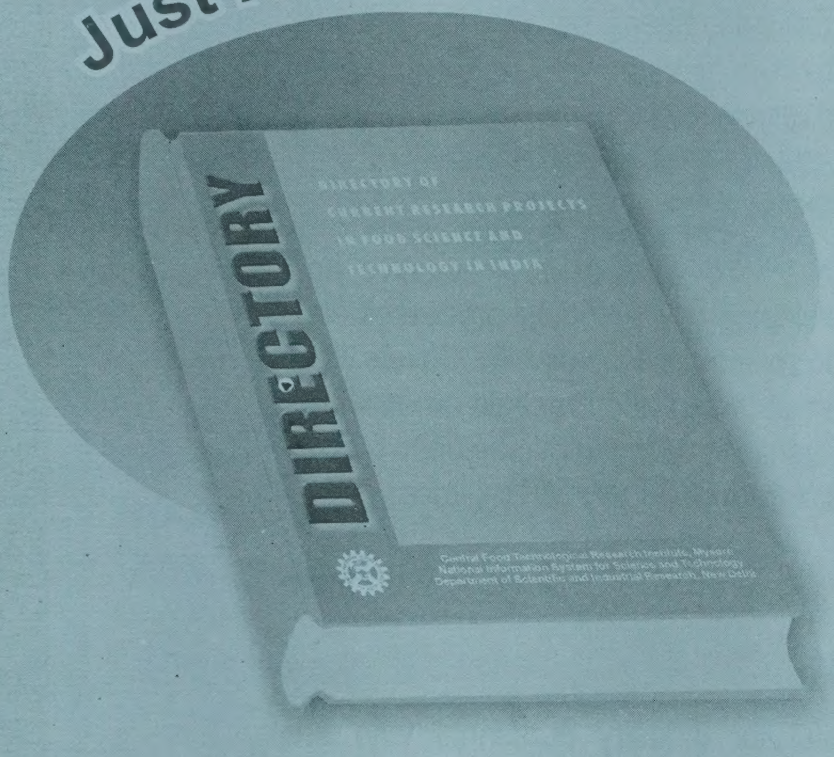

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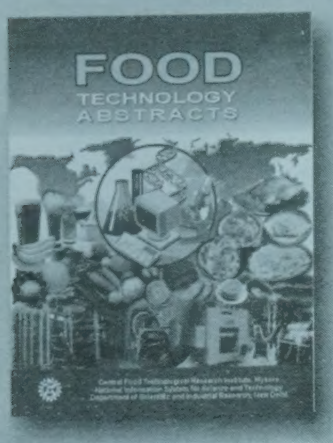

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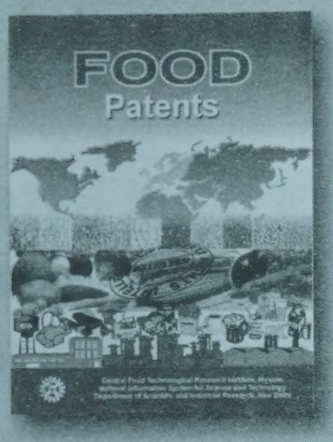

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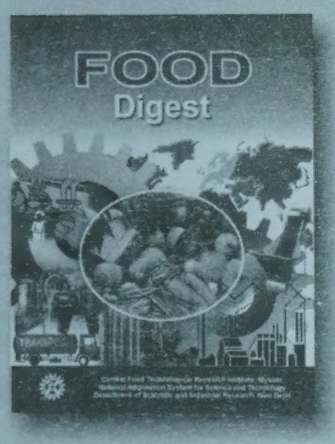

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